BIOLOGICAL BULLETIN

OF THE

Woods Hole. **Marine Biological Laboratory**

WOODS HOLE, MASS.

Editorial Staff

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GEORGE T. MOORE—*The Missouri Botanic Garden.*

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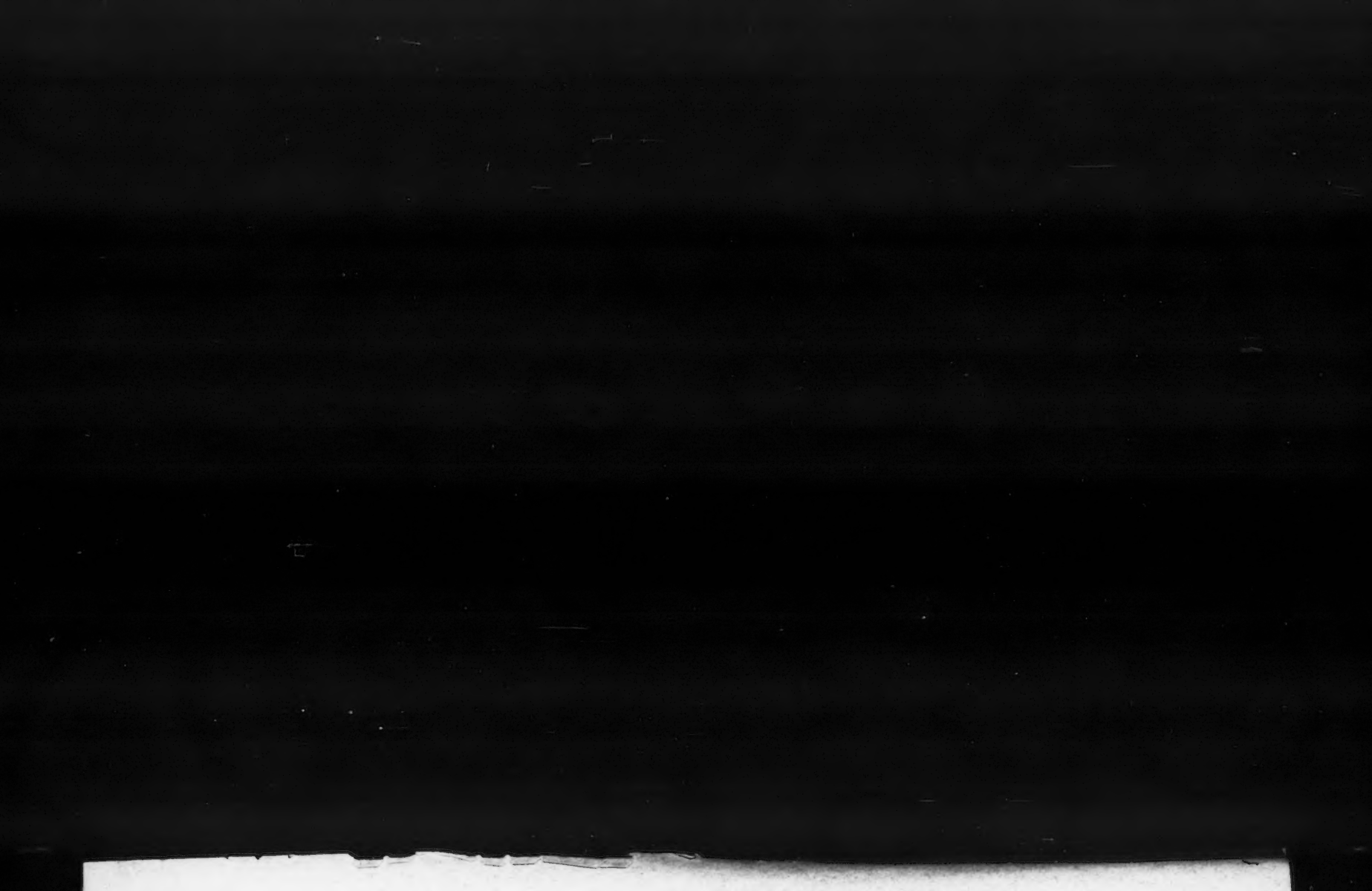

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BIOLOGICAL BULLETIN

STUDIES ON THE INFLUENCE OF INANITION ON THE DEVELOPMENT AND THE DURATION OF LIFE IN INSECTS.

STEFAN KOPEĆ.

GOVERNMENT INSTITUTE FOR AGRICULTURAL RESEARCH, PULAWY, POLAND.

WITH 3 TABLES.

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The experiments were performed on the caterpillars of the common moth *Lymantria dispar* L. Each experiment consisted of several separate broods. The caterpillars deriving from one brood were divided in two parts and put in two jars, one part was subjected to starvation, the other, daily fed, was used as a control. The inanition applied in these studies was total but intermittent, the caterpillars being totally deprived of food during certain days and fed *ad libitum* during the remaining. The distribution of these days was different in different series of experiments, as may easily be seen from tables 1 to 3. The obtained pupæ being kept each separately, it was possible to study the influence of starvation not only on the duration of the stage of caterpillars, but also on that of each chrysalis and each moth.

In the chief experiments (series *A* to *F*) the whole material consisted of 1239 starved caterpillars, and 843 specimens for control; 547 pupæ were obtained from the caterpillars subjected to starvation and 763 from control larvæ; they produced 476 "starved" and 683 control moths (Cf. Table I.). The increased

[1] Paper from the Laboratory of Experimental Morphology., cf. *Mém. de l'Institut Nat. Polonais d'Économie Rurale à Pulawy*, Vol. 1, 1921.

TABLE I

Onset of Experiments.	Starvation Applied to:	Number Referring to Each Brood.	Number of Pupæ.				Number of Moths.				Weight of Pupæ in Mg.							
			Starved.		Control.		Starved.		Control.		♂♂.				♀♀.			
											Starved.		Control.		Starved.		Control.	
			♂♂.	♀♀.	♂♂.	♀♀.	♂♂.	♀♀.	♂♂.	♀♀.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual. Fluctuations.	Average.
Since hatching from eggs.	*Series A.* Starvation lasting one day every second day (+−+−+−) applied during the whole larval life.	1	10	14	21	35	7	11	18	28	180–394	252.7	262–524	383.7	433– 935	686.2	697–1277	1006.3
		3	9	12	16	15	9	11	15	15	302–421	364.0	410–565	507.1	629–1158	963.0	872–1570	1261.6
		4	15	16	15	30	12	14	12	24	215–480	304.6	180–471	376.8	612–1181	907.1	420–1264	870.6
		11	10	13	35	38	10	12	30	31	225–317	271.3	184–572	430.5	348– 852	698.5	485–1605	1057.4
	Series B. The same starvation applied only during the first 20 days of larval life.	2	10	14	14	24	8	11	12	22	222–433	345.3	271–471	394.4	755–1382	1087.7	611–1535	1175.0
		9	12	8	15	16	12	8	12	11	395–565	439.6	280–556	388.0	470–1557	1097.2	538–1354	1030.8
		12	10	10	17	16	8	10	14	10	291–502	416.2	275–514	399.3	563–1504	1148.8	598–1521	1073.1
Since the last moult but one.	*Series C.* Starvation lasting one day every third day (++−++−)	34	15	9	12	9	14	8	12	9	340–425	366.8	468–533	496.1	760–1015	876.0	1257–1465	1341.6
		35	18	11	18	8	14	10	18	7	273–395	333.7	447–586	509.1	414– 793	577.8	624–1587	1273.5
		36	16	10	16	12	10	10	14	12	232–355	314.2	344–511	444.2	322–1280	833.6	996–1081	1034.5
		37	12	12	16	10	12	12	14	10	247–372	303.3	335–507	409.0	620– 917	814.2	820–1230	1042.6
		38	15	18	16	16	12	14	16	16	255–369	306.6	419–557	466.2	444–1063	733.6	850–1284	1074.8
		39	9	9	11	15	7	8	9	12	200–396	291.7	373–537	447.7	375– 739	581.1	734–1576	1131.8
	Series D. Starvation lasting one day every second day (+−+−+−)	28	11	8	13	9	9	8	12	8	163–274	212.2	298–454	399.1	530– 782	665.8	962–1293	1146.3
		29	13	13	15	15	13	12	15	12	171–212	191.5	431–490	452.4	363– 590	475.0	745–1320	1115.0
		30	16	9	15	10	16	7	15	10	185–302	242.5	401–567	445.4	290– 638	469.3	867–1285	1052.4
		31	12	12	14	14	12	10	13	14	185–279	232.0	409–530	477.4	382– 963	595.7	1147–1565	1329.4
		32	12	12	12	13	12	9	12	12	145–298	230.1	279–500	419.5	373–1085	689.6	663–1352	1176.9
		33	12	10	10	10	9	8	9	9	155–195	168.6	343–537	439.1	347– 695	489.4	808–1350	1150.3
	Series E. Starvation lasting two days every second day (+−−+−−)	40	6	8	7	14	6	6	6	14	121–207	154.0	422–552	480.2	156– 283	230.8	715–1371	1138.0
		42	4	6	10	11	3	6	10	10	118–168	135.6	391–427	411.6	131– 378	269.1	705–1302	1059.3
		45	6	6	13	10	6	4	13	10	100–131	112.5	355–540	446.8	172– 421	318.2	844–1355	1088.3
	Series F. Starvation lasting two days every third day (++−−++−−)	46	10	8	14	16	6	7	14	14	165–272	173.7	365–525	443.4	299– 590	428.1	1112–1860	1349.5
		47	8	10	8	16	8	10	6	14	158–210	182.5	445–602	516.2	355– 486	418.0	922–1528	1130.7
		48	8	10	14	14	7	8	14	14	155–257	172.8	330–556	505.7	289– 473	395.2	913–1052	998.6

mortality of the starved caterpillars ought not to be ascribed first of all to the emaciating influence of inanition, the chief cause of this mortality is the moult. Directly after moulting the animal takes very much food, as during these processes it does not eat at all and only digests its own substances. Therefore when the fasting day falls on the period succeeding moult the caterpillars are often unable to resist starvation. The mortality of chrysalids, on the contrary, was identical in starved and in control specimens. The willow leaves on which the caterpillars were fed were always of the same variety and of the same freshness. The conditions of space, light and moisture were identical in all jars during the whole t me of observations and the temperature was from 16° to 19° C. Food was put in great superfluity into control jars every morning and into those containing starved material during the feeding days and it preserved its freshness the whole day. All experiments were performed during one season (1920).

1. The Influence of Inanition on the Duration of Separate Developmental Stages.

For the experiments of series *A* caterpillars belonging to four different broods were used, from 1 to 6 hours after hatching. These caterpillars were deprived of food every second day, in the remaining days they were fed *ad libitum*. Starvation lasting one day and applied every second day may be designated by the symbol $+-+-+-$. Table II. records the limits of individual fluctuations in the duration of life of the caterpillars and chrysalids belonging to each brood, as well as the average duration of life in starved and in control specimens of the two sexes. Table III. finally shows the average differences of the duration of larval and of pupal life of individuals deprived of food for each brood separately, calculated in percentages of the average duration of the larval or of the pupal stages of control specimens of the same brood and the same sex. From these tables we see that the life of caterpillars subjected to such starvation was considerably prolonged. In all lots without exception the longest-lived control caterpillars underwent pupation earlier than the shortest-lived starved specimens of the same brood and sex. (Cf. Table II. *Series A*). If we take the average of the averages of all broods of series *A*, a quantity which I shall call "average of broods,"

TABLE II

Onset of Experiments.	Starvation Applied to:	Number Referring to Each Brood.	Number of Days of Larval Life from Onset of Experiment till Pupation.								Number of Days of Pupal Life.							
			♂♂.				♀♀.				♂♂.				♀♀.			
			Starved.		Control.		Starved.		Control.		Starved.		Control.		Starved.		Control.	
			Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.	Limits of Individual Fluctuations.	Average.
Since hatching from eggs.	*Series A.* Starvation lasting one day every second day (+−+−+−) applied to during the whole larval life.	1	62–80	70.7	42–49	43.8	75–81	78.0	43–58	46.0	11–16	14.7	19–23	22.2	10–12	11.0	17–21	19.8
		3	63–74	67.6	42–47	45.0	75–81	78.5	45–55	48.0	15–18	16.6	22–25	23.4	10–12	11.0	20–22	20.8
		4	63–84	71.8	44–49	45.7	71–81	76.5	44–68	49.2	12–20	16.0	22–25	23.8	10–13	11.2	17–21	20.2
		11	62–67	64.5	43–49	45.3	75–78	76.0	44–59	48.2	14–19	16.5	21–26	23.2	11–12	11.6	19–22	20.0
	Series B. The same starvation applied only during the first 20 days of larval life.	2	56–70	61.1	43–55	45.8	60–67	63.0	44–59	48.6	16–21	19.2	21–25	23.7	15–17	16.0	18–22	20.4
		9	57–69	62.5	44–68	49.4	63–72	68.3	45–68	52.5	17–21	19.0	18–24	23.0	13–17	15.3	16–21	20.1
		12	63–77	69.3	44–69	54.4	64–71	67.6	44–64	51.6	14–20	17.8	16–24	21.2	14–18	16.0	18–22	20.6
Since the last moult but one.	*Series C.* Starvation lasting one day every third day (++−++−)	34	21–23	22.2	18–20	18.8	23–37	28.1	19–20	19.6	22–23	22.2	22–24	23.1	16–23	19.8	20–22	21.0
		35	21–24	22.4	18–20	18.8	26–36	30.0	20–27	21.5	21–24	22.5	22–24	23.2	16–23	19.8	20–22	21.0
		36	20–28	22.5	18–21	19.6	24–36	29.4	20–22	20.6	21–22	21.6	21–23	22.2	16–21	19.0	19–22	20.1
		37	21–28	23.3	19–21	19.7	23–31	26.2	20–21	20.4	20–23	21.5	20–25	22.4	18–20	19.2	20–21	20.6
		38	20–22	21.4	18–22	19.5	22–28	23.8	20–22	20.6	22–24	23.2	23–25	24.0	19–20	19.7	20–22	20.7
		39	21–32	23.3	19–20	19.8	22–35	26.3	19–26	21.1	21–23	22.1	21–24	22.8	18–20	19.3	19–21	20.2
	Series D. Starvation lasting one day every second day (+−+−+−)	28	24–30	27.0	16–22	18.0	26–29	26.8	17–21	19.2	20–22	21.3	22–23	22.6	17–22	19.8	20–22	21.0
		29	25–27	26.0	16–19	17.8	26–30	28.0	18–22	19.4	21–23	22.0	22–24	22.8	18–20	19.3	20–21	20.7
		30	23–25	24.0	17–19	18.0	23–39	29.5	16–20	18.2	21–22	21.5	23–25	23.8	16–20	18.4	20–21	20.6
		31	24–25	24.5	17–20	18.5	23–39	28.8	18–23	20.5	21–23	22.0	21–24	23.0	19–22	20.5	21–23	21.7
		32	23–33	26.3	17–22	19.1	23–38	30.2	18–25	20.1	20–24	22.0	21–24	22.9	16–21	19.0	19–22	20.3
		33	24–33	29.0	18–22	20.1	27–43	35.1	20–23	21.2	21–22	21.6	22–24	23.2	14–20	17.3	20–23	21.0
	Series E. Starvation lasting two days every second day (+−−+−−)	40	27–32	29.3	18–19	18.4	30–46	37.0	18–23	19.5	16–21	18.6	22–24	22.8	13–15	14.3	20–22	21.0
		42	25–38	31.2	18–20	18.8	28–45	37.0	18–22	19.3	17–19	18.0	22–23	22.4	12–15	13.6	19–21	19.7
		45	26–35	31.3	17–21	18.3	28–48	37.1	19–21	19.8	16–19	17.3	22–25	23.8	13–16	14.7	19–21	20.3
	Series F. Starvation lasting two days every third day (++−−++−−)	46	22–38	28.9	18–20	18.7	33–39	36.5	18–24	19.8	17–21	19.8	21–23	22.4	14–16	15.1	19–22	20.2
		47	32–34	33.0	19–24	20.8	37–39	38.0	20–24	21.7	18–20	19.0	23–24	23.3	13–16	14.5	20–21	20.4
		48	21–39	27.1	18–19	18.1	29–37	33.2	18–20	19.0	17–21	19.4	23–25	23.8	15–18	16.5	20–21	20.6

TABLE III

		Experiment Begun Since Hatching from Eggs.									Experiment Begun Since the Last Moult But One.																			
		Series A Starvation Lasting One Day Every Second Day (+−+−+−) Applied During the Whole Larval life.					*Series B.* The Same Starvation Applied to Only During the First 20 Days of Larval Life.				*Series C.* Starvation Lasting One Day Every Third Day (++−++−).							*Series D.* Starvation Lasting One Day Every Second Day (+−+−+−).							*Series E.* Starvation Lasting Two Days Every Second Day (+−−+−−).				*Series F.* Starvation Lasting Two Days Every Third Day (++−++−).			
		Number Referring to Each Brood.				Average of Broods.	Number Referring to Each Brood.			Average of Broods.	Number Referring to Each Brood.						Average of Broods.	Number Referring to Each Brood.						Average of Broods.	Number Referring to Each Brood.			Average of Broods.	Number Referring to Each Brood.			Average of Broods.
		1	3	4	11		2	9	12		34	35	36	37	38	39		28	29	30	31	32	33		40	42	45		46	47	48	
Average prolongation of life of the starved caterpillars in per cent. of the average duration of larval life of control specimens of the same brood and sex, from the time of onset of experiment till pupation.	♀♀	61.4	50.2	57.1	42.3	*52.7*	33.4	26.5	27.3	*29.0*	18.0	19.1	14.7	18.2	9.7	17.6	*16.2*	50.0	46.0	33.3	32.4	37.6	44.2	*40.5*	59.2	65.9	71.0	*65.3*	54.5	58.6	49.7	*54.2*
	♂♂	69.5	63.5	55.4	57.6	*61.5*	29.2	30.0	31.0	*30.0*	43.3	39.5	42.7	28.4	15.5	24.6	*32.3*	39.5	44.3	62.0	40.4	50.2	65.5	*50.3*	89.7	91.7	87.3	*89.5*	84.3	75.1	74.7	*78.0*
Average abbreviation of life of the "starved" pupæ, in per cent. of the average duration of pupal life in control specimens of the same brood and set.	♀♀	33.7	29.0	32.7	28.8	*31.0*	18.9	17.3	16.0	*17.4*	3.8	3.0	2.7	4.0	3.3	3.0	*3.3*	5.7	3.5	9.6	4.3	3.9	6.8	*5.6*	18.4	19.6	27.3	*21.7*	11.6	18.4	18.4	*16.1*
	♂♂	44.4	47.1	44.5	42.0	*44.5*	21.5	23.8	22.3	*22.5*	5.7	5.7	5.0	6.7	4.8	4.4	*5.3*	5.7	6.7	10.6	5.5	6.4	17.6	*8.7*	31.9	30.9	27.5	*30.1*	25.2	28.8	19.9	*24.6*
Average weight of the "starved" pupæ in per cent. of the average weight of control chrysalids of the same brood and set.	♀♀	65.5	71.7	80.9	63.0	*70.2*	87.5	113.2	104.2	*101.6*	73.9	65.5	70.7	74.1	65.7	65.1	*69.1*	53.1	42.3	54.4	48.5	54.8	38.3	*48.5*	32.0	32.9	25.1	*30.0*	39.1	35.3	34.1	*36.1*
	♂♂	68.1	76.3	104.1	66.0	*78.3*	92.5	106.4	107.0	*101.9*	65.2	45.3	80.5	78.0	68.2	51.3	*64.7*	58.0	42.6	44.5	44.8	58.5	42.5	*48.4*	20.2	25.4	29.2	*24.9*	31.7	36.9	39.5	*36.0*

we obtain for male specimens a prolongation of larval life of 52.7 per cent. for the female of 61.5 per cent. Cf. Table III. As the processes of pupation were checked every 24 hours and the caterpillars were taken for the experiments from 1 to 6 hours after their hatching from eggs, the error concerning duration of the larval stage could have in no case exceeded 30 hours.

The duration of the pupal stage is influenced by the starvation of caterpillars in an essentially different manner, viz, the chrysalids which have developed from starved caterpillars undergo transformation into adult moths far earlier than control pupæ, but the abbreviation of the pupal stages is smaller than the prolongation of larval life. This abbreviation amounted in the "averages of broods" in males to 31.0 per cent. in females to 44.5 per cent. of the average duration of pupal stage of control specimens. Pupation having been controlled every 24 hours, emergence of moths every 12 hours, the error in estimation of the duration of the pupal stage could not exceed 36 hours.

The moths of *Lymantria dispar* L. do not take any food in their imaginal stage. Control males lived as moths in separate lots from 1 to 8, from 5 to 8, from 2 to 8 and from 1 to 8 days and the "starved" specimens from 4 to 5, from 5 to 11, from 2 to 8 and from 1 to 6 days. Normal females lived from 3 to 13, from 1 to 10, from 1 to 12 and from 2 to 14 days, those derived from starved caterpillars from 5 to 6, from 3 to 10, from 7 to 10 and from 6 to 10 days. Comparing the mean values of the duration of life of the normal and of the "starved" moths we obtain in most cases a certain prolongation of life of the moths derived from starved caterpillars (+) though rarely a certain abbreviation (−). This prolongation or abbreviation calculated in days amounts in separate broods in the males to + 0.3, + 2.5, + 0.1 and −0.1 days, in the females to −2.4, + 0.3, + 2.8 and + 2.1 days. We see that starvation of caterpillars has no distinct effect on the duration of the imaginal stage. Emergence and death of moths having been checked every 12 hours, the error in the estimation may attain only 24 hours. The moths of the two sexes experimented upon both control and "starved" have never been allowed to mate.

If we take into consideration the behavior of the "starved" moths as well as the fact that the abbreviation of the pupal

period was smaller than the prolongation of the larval stage, we must draw the conclusion that total deprivation of food of caterpillars every second day has a considerable positive effect on the total duration of their life from hatching until death. The prolongation of the whole developmental period amounts here in the average of broods in males to 16.5 and in females to 20.4 days, or in percentages relative to the duration of life of control specimens in males to 24.2 per cent. and in females to 30.0 per cent.

The above results find complete confirmation in my farther comparative experiments in which inanition of various intensity was applied (series *C*, *D* and *E*). In these experiments caterpillars were used from 1 to 12 hours after their last moult but one. In series *C* food was administered two days, the third day the insects were starved (starvation lasting one day every third day $++-++-++-$). In series *D* the animals were again deprived of food every second day (starvation lasting one day every second day $+-+-+-$) and the caterpillars of series *E* were deprived of food two days and ate only every third day (starvation lasting two days every third day $+--+--+--$). From the absolute figures of Table II. as well as from the percentages in Table III we may infer that the duration of the larval period undergoes also, in these starved animals considerable prolongation (cf. analogous results obtained in silk-worms by Kellogg and Bell, '04 *b*), simultaneously with abbreviation of the pupal stage. These changes become more and more remarkable in proportion as inanition increases, they are larger in series *D* than in series *C* and the largest in series *E* in which the fasting days were the most numerous. Also in these comparative experiments the prolongation of the larval life was greater than the abbreviation of the pupal period, while the duration of the imaginal life remained unchanged. Hence it follows that the total duration of the life of the insects from the beginning of the experiment till death of the moth, is more and more increased in proportion as inanition increases within the limits of the experiments. The duration of development from the last moult but one till the emergence of the moth underwent in the average of broods a prolongation (calculated in percentages of the average duration of such control periods) which in the males

amounted to 5.6 per cent. in series *C*, to 15.1 per cent. in series *D* and to 16.8 per cent. in series *E*; in the females the prolongation was 13.3 per cent. in series *C*, 20.2 per cent. in series *D* and 28.6 per cent. in series *E*.

In series *F* the caterpillars were fed during two days and deprived of food during the next two. (Starvation lasting two days every third day $++--++--$). Notwithstanding a different distribution of the feeding and fasting days, the quantitative relation of these days was the same as in series *D* to which starvation lasting one day every second day was applied ($+-+-+-$); in both experiments the caterpillars experimented upon were of the same age. It nevertheless turned out that the prolongation of life was much greater in caterpillars of series *F* than in specimens belonging to series *D*. (Cf. Table II. and III.) In series *F* it amounted in the average of broods in the males to 54.2 per cent. of the average of the life of control caterpillars from their last moult but one, and in the females to 78.0 per cent while in series *D* the corresponding numbers were 40.5 per cent. in males and 50.3 per cent. in females. The abbreviation of the pupal period calculated similarly was in series *F*, in males 16.1 per cent of the duration of life of control chrysalids and in females 24.6 per cent. while in series *D* the life of male pupæ underwent abbreviation of 5.6 per cent. and of the female 8.7 per cent. We see that changes of the duration of the larval and of the pupal stages induced by inanition depend not only on the mutual quantitative relation of the fasting and feeding period, but also on the distribution of these periods. The organism responds by more energetic reaction to longer, though less frequent, fasting intervals than to more frequent but shorter periods of starvation.

3. Inanition and its Bearing on the Physiology of Insect Metamorphosis.

When we try to discuss the foregoing results on the basis of hitherto existing references in the literature, we meet certain discrepancies which, however, may be cleared up by consideration of the physiology of animal metamorphosis.

Kellner ('87) fed caterpillars of the silk-worm during their whole life on insufficient quantities of leaves, and obtained a

short prolongation of the larval stage. Analogous experiments were afterwards made by Kellogg and Bell ('04 *a* and *b*). Pictet ('05) came to the conviction that the larval stage is prolonged and the pupal stage shortened by feeding caterpillars on plants containing few food-stuffs, but that the conjoint periods of development do not undergo any changes. A prolongation of larval life caused by inadequate food was lately determined by Northrop ('17) Tangl. ('09*a*), finally, maintains that the larvæ of flies reared on pure egg-white which they refused to take underwent transformation approximately a week later than normally. These results are, in general, concordant with my observations on starved caterpillars. On the other hand, the observations of Krizenecky ('14) and of Szwajsówna ('16) on the larvæ of *Tenebrio molitor* which underwent metamorphosis earlier when totally starved contradict the above determinations. A similar discrepancy may also be remarked in analogous investigations on amphibians. While Barfurth (87) and others have ascertained that starvation of amphibians causes acceleration of their metamorphosis, other investigators induced retardation of these processes by the administration of poor food to tadpoles. It has been emphasized by Wolterstorff ('96), Morgan ('07), Kaufman ('18) and others that in amphibians the influence probably differs in different developmental stages of the tadpoles used for starvation. The former of the above mentioned investigators deprived large tadpoles of food, in part shortly before their transformation, while the others used far younger specimens. Similarly the experiments of Kellner and the following authors and also my own were made on young caterpillars while the observations of Krizenecky and of Szwajsówna refer to older larvæ. In another experimental study on starved tadpoles (Kopeć, *22a* and *b*) I succeeded in confirming the above views of Wolterstorff, Morgan and others. My experiments showed that metamorphosis of the tadpoles to which starvation was applied before the fiftieth day of their development undergoes retardation, where as transformation is accelerated when tadpoles are starved after the sixty fifth day of their life. In this study on caterpillars similar behavior was also noted in one and the same material of female larvæ. It turned out that the caterpillars to which total starvation has been applied approximately since

the seventh day after their last moult underwent pupation in three different broods after from 7 to 13, from 7 to 16 and from 7 to 15 days (or on the average after 10.6, 10.8 and 10.0 days), whereas the control specimens of these broods become chrysalids as early as after from 6 to 12, from 6 to 12 and from 5 to 12 days (or on the average after 8.2, 9.3 and 8.6 days). On the contrary the larval life of the females of the same broods in which starvation began approximately from the tenth day after the last moult was much shorter than that of control larvæ, as pupation occurred here from 4 to 8, from 5 to 8 and from 4 to 8 days (or on the average after 5.5, 6.6 and 6.8 days), in control specimens from 5 to 10, from 6 to 10 and from 7 to 9 days (or on the average after 7.4, 7.6 and 8.1 days). Hence follows: (1) The character of the influence, whether accelerating or retarding, exerted by starvation on the processes of metamorphosis depends on the period of life or the developmental stage on which the factor begins. (2) This moment may be accurately determined by means of experiment, viz, in the females of *Lymantria dispar* L. In my cultures the critical moment during which the influence of inanition delaying metamorphosis is changed into an accelerating one fell on the period between approximately the seventh and the tenth day after the last moult.

An essential explanation of such different behavior of caterpillars may be found in my former studies on the importance of the brain for insect metamorphosis (Kopeć, '17 and '22*c*). It was shown that the female caterpillars of this moth, deprived of brain the seventh day after their last moult, live far longer than control animals, but they die without undergoing transformation. On the other hand, the removal of the brain from caterpillars the tenth day after their last moult has no influence either on the processes of pupation or on the emergence of moths. From various experiments supported by observation on control material it follows that the brain plays here most probably the rôle of an organ of internal secretion. From these results we may infer that by depriving caterpillars of food since the tenth day after their last moult we afford exceptionally favorable conditions for metamorphosis, viz, the substance or substances already produced by the brain in sufficient quantities find in such starved organisms less material which ought to be transformed

in time. The prolongation of the larval period in younger caterpillars starved before the seventh day after the last moult may be explained, on the contrary, by impeded secretoric function of the brain or by certain anomalies of its activity in starved organisms.

If the processes occurring in the pupa were a continuation of those taking place in the caterpillar and leading to pupation, the abbreviation of the pupal stage in starved specimens could not be reconciled with the above explanation. But these processes differ not only energetically as it has been shown by Tangl ('09*b*). The processes of pupation may morphologically be reduced to the histolysis of almost all larval tissues into a homogeneous mass which fills up the pupal body, whereas the transformation of the pupa into the fully-developed insect consists in the developemnt of the so-called imaginal discs, *i.e.*, of small accumulations of cells. In my present experiments (series *B*) I succeeded moreover in establishing certain physiological characters which point to another difference. In series *B* the caterpillars were deprived of food every second day for 20 days after their hatching from eggs, *i.e.*, at a time when there are not even any traces of histolytic processes in the larval body. From the twenty-first day till the end of the larval period the animals were fed every day and underwent the normal number of moults. From the appertaining items on Tables II. and III. we see that the caterpillars belonging to this series not only remarkably delayed the term of their pupation, but also that the duration of the pupal stage was considerably shortened. This abbreviation amounted in the average of broods to 17.4 per cent. of the average duration of life of normal pupae in males and to 22.5 per cent. in females. Consequently, the evolutive processes characteristic of the pupa are not a continuation of those changes which are characterized by histolysis of larval tissues. We see that by temporarily starving young caterpillars (Series *B*) we may segregate these processes and prove that processes of the development of the imaginal discs may begin far earlier, *i.e.*, soon after the hatching of the caterpillar from the egg. It ought therefore to be inferred that the brain has two separate functions in normal conditions, (1) it causes histolysis of larval tissues, (2) it delays the evolution of imaginal discs. During inanition of caterpillars the influence of the brain hindering the development of imaginal discs

is decreased, owing to its lowered function. The starved chrysalids therefore begin their pupal stage when the discs are better developed and this causes acceleration of the development of the imaginal body and hence abbreviation of the pupal life.

The assumption that the larval brain exerts two different influences is neither astonishing nor incomprehensible in respect to the well-known data concerning the physiology of organs of internal secretion. The final development of the imaginal discs and their definitive differentiation sets in before pupation or even in the pupa, *i.e.*, at a time when almost all larval tissues are undergoing histolysis or have undergone it. Therefore it ought to be admitted that during this period the influence of the brain hindering the development of embryonal discs normally becomes annuled by certain processes which are unknown to us. It is very probable that the development of imaginal discs and the histolysis of the larval tissues become, at least towards the end of larval life, physiologically correlated with each other. In the contrary case it could be hardly understood why the caterpillars deprived of their brain the seventh day after their last moult do not, it is true, exhibit any histolytical changes in their tissues, but the imaginal discs contained in their body do not undergo final growth and differentiation. (Kopeć, '17 and '22 *c*). Indeed, if there were no such correlation, the imaginal discs of the caterpillars deprived in that period of their brain (and therefore of the organ retarding their evolution) ought to develop the organs of the imago in spite of the absence of histolytical processes in the larval body.

In contrast to my experiments on the starvation of caterpillars, Loeb and Northrop ('17) have lately convinced themselves that each of the separate stages of life in Drosophila occurs more slowly in lower temperature and more quickly in higher ones. The changes of temperature were applied by the mentioned authors during the whole development of the animals, while in my experiments the factor of inanition had a direct influence only on the processes taking place in caterpillars, as the chrysalis does not take food in normal conditions either. Loeb and Northrop obtained the same changes of the duration of the larval and of the pupal stage first of all owing to changed celerity of metabolism in the larva as well as in the pupa. Experiments in which

lower or higher temperatures should be applied only to larvae, the chrysalids being kept in normal conditions, might solve the problem as to whether and to what degree the changed temperature has an influence not only on the celerity of metabolism, but also on the supposed function of the brain.

The attempts hitherto made to explain the metamorphosis of insects refer to the last stage of the processes. The appearance of phagocytosis, of degeneration, of asphyxia and of other "causes" of transformation is not yet elucidated. My experiments on the function of the larval brain as well as the discovery of tyrosinase in caterpillars and chrysalids made by Dewitz ('05 and '16) and confirmed by Steche and Waentig ('13) together with the present results on the starvation of caterpillars may lead to a better knowledge of the cause of insect metamorphosis. But my "secretory" theory of metamorphosis will not be well grounded until we succeed in finding in the structure of the brain an adequate base for the theory, *i.e.*, until certain specific changes in the brain not only during pupation, but also during moults will have been ascertained. A great support might be afforded by positive experimental results on transplantation of brains, or on injections of extracts of this organ into brainless specimens. The latter experiments only would be able to dispel every doubt in regard to the validity of my previous conclusion that the indubitable influence of the brain on metamorphosis is due to an internal secretion of this organ.

Deegener ('09) lastly considers the imaginal form to be phylogenetically older than the larval form, which developed secondarily from the fully developed insect owing to numerous secondary life conditions "unter fortgehender Retardation der Entwicklung imaginaler Organe" (p. 11). I think that my experiments of series *B* distinctly prove that in normal circumstances the development of the imaginal discs is retarded during larval stages. I cannot deny that in the light of these results the supposed retardation of the development of the imaginal organs gains an experimental base, at least in ontogenetic evolution.

3. Inanition and Certain Problems of Growth.

In this chapter I take into account only the final stages of growth of caterpillars expressed by the weight of new-formed

pupæ. The chrysalids were weighed from 1 to 24 hours after pupation, control weighings having proved that the weight of pupæ decreased in my broods during this period only from 0.18 per cent. to 0.33 per cent. The limits of fluctuations in the weight of pupæ are recorded in Table I., together with their average values. On Table III. we see moreover the average weight of "starved" chrysalids of each brood calculated in percentages of the average weight of control chrysalids of the same sex and brood.

On comparing the data of series *C*, *D* and *E* we see that the weight of chrysalids decreases more and more in proportion as the number of fasting days increases. Assuming in general that the number of feeding days in separate series corresponds to the quantity of food which has been taken, we may say that the weight of pupæ is in direct relation to the quantity of food consumed. If we also take into consideration the data of series *F* in comparison to analogous items of series *D*, we draw the conclusion that the average decrease of chrysalids due to inanition of caterpillars is larger when the feeding intervals are longer though less frequent. As it was pointed out, the prolongation of the stage of caterpillar and the abbreviation of that of pupa increases in proportion as more and more intense starvation has been applied. Consequently, the weight of the pupæ the caterpillars of which had been starved is in inverse relation to the prolongation of the larval period as well as to the abbreviation of the stage of chrysalids. Adopting the ratio of the pupal weight to the duration of larval life as the approximative measure of the rate of growth, we can infer from Tables I. to III. that this rate decreases in proportion as more and more intense starvation has been applied and in relation to the distribution of the feeding and fasting days (Series *F* and *D*). The above-stated principles refer to separate series of experiments but not to separate specimens, either control or starved, in one brood. I have often observed that although the processes of transformation lasted in the control or in the starved material in every brood several days, the heaviest and the lightest caterpillars underwent pupation the same day, sometimes the first and sometimes not until the last day, although the one was two- or threefold heavier than the other. The same may be said as to the duration of pupal

life in regard to the weight of separate chrysalids of one brood.

The problem arises whether the capacity to grow is checked by age of the animal. Such limits in rats have been adopted by Aron ('12) and lately by Jackson and Stewart ('20), in contrast to Osborne and Mendel ('14) who are inclined to the opinion that the capacity to grow is exhausted by mere growth, without regard to the factor of time. In series *B* of my experiments the caterpillars, deprived of food intermittently for 20 days since their hatching, weighed the twenty-first day in separate broods on the average only 7.6, 3.6 and 5.0 mg., while control individuals of the same broods had the mean weight of as much as 36.9, 45.5 and 40.3 mg. The starved specimens fed daily since the twenty-first day attained and partly exceeded in time the weight and size of control caterpillars (cf. weights on Table I. and III.). As in this series the processes of metamorphosis of originally starved caterpillars were retarded and as, on the other hand, caterpillars have grown until the end of their larval life, it ought to be inferred that in these animals the capacity to grow is not suppressed at a period at which caterpillars normally cease to grow. Moreover, it may be concluded that pupation also may take place much beyond the age at which the control specimens undergo pupation. But the inference that the capacity may not at all be limited by the age of animals ought not to be drawn from such results. It is possible that the rats experimented upon by Mendel and Osborne as well as my caterpillars would lose the capacity to obtain the weight and size of control specimens if they had been kept at maintenance or starved even a few days longer.

4. Adaptation of Organisms to Starvation. Some Remarks on the Problem of Death.

The weight of pupæ from series *A* (in which starvation lasting one day every second day, $+-+-+-$, was applied to caterpillars during the whole larval life) is evidently different from that of series *D* in which older specimens after their last moult but one were subjected to the same starvation (cf. Table I.). We see that organisms may in time get accustomed to the detrimental effects of starvation, which prevent the animal from attaining its normal weight: the comparison of the limits of individual fluctuations shown on Table I. points to the conclusion

that the chrysalids of series *D* which have been deprived of food only during the half of their larval life are lighter than those from series *A* the caterpillars of which have been starved during their whole larval life. From Table III. we see that the male pupæ from series *D* weighed in the average of broods 48.5 per cent., the female pupæ 48.4 per cent. of the average weight of the control chrysalids, whilst the male chrysalids from series *A* weighed as much as 70.2 per cent., the females 78.3 per cent.

As the prolongation of the larval period was much greater in series *A* than in series *D* (cf. Table I.), it might be supposed that the caterpillars of series *A* are heavier owing to the circumstance that they lived longer, and therefore could take and digest food during a longer period. But the following argument contradicts this opinion. At the outset of the experiment in series *D* the caterpillars weighed in the average of broods 94.5 mg., the male specimens underwent pupation in the average of broods after 26.1, the female after 29.7 days. If we take as starting point for series *A* the day on which the caterpillars of this series had an analogous weight, which in the average of broods amounted to 93.5 mg., the duration of larval life in this series from this day till pupation was in the average of broods 27.6 in males and 36.2 in females. We see that when the starting point had been made uniform the duration of farther larval life in series *A*, especially in males, is almost identical with that in series *D*. In other words, the specimens of series *A* attain considerably greater weight than those of series *D* during approximately the same period. The ratio of the produced number of milligrams of body-weight to the total number of days of the period during which the larvæ had been deprived of food being considered as rate of growth, we may calculate that the rate of growth amounts in the average of broods in series *A* to 7.4 in starved males and to 19.8 in females, whilst in series *D* this quantity attains only 4.5 in starved males and 15.8 in females. It follows that the starved organism may in time get accustomed to the metabolism of inanition, *i.e.*, the ratio of assimilation to disassimilation becomes during long starvation changed in favor of the organism.

By histological research I have convinced myself that caterpillars which died from starvation contain no adipose tissue. In a certain contrast to my observations, Bialaszewicz ('19) draws

from his experiments on the starvation of leeches as well as from the papers by several authors on other animals the conclusion supported by numerous grounds that fat has no great importance in the hunger metabolism of cold-blooded animals, but that it undergoes only small reduction. The farther investigations of Bialaszewicz will undoubtedly show whether my supposition based on histological research is right, or they may explain the cause of this discrepancy which possibly consists in the physiological capacity of caterpillars to digest their store of adipose tissue during their frequent moults.

In contemporary research the cause of natural death is regarded as caused by the accumulation of detrimental products of normal metabolism which cannot be removed from the multicellular organism. This view is not at all proved; it is based first of all on the known investigations of Woodruff on the infusoria whose conclusions are in discrepancy with the results obtained by Viewegerowa and Vieweger ('22) who by methodically exact research proved that the products of metabolism have no great importance on the development of Colpidium and that the divisions are hindered in unchanged surroundings first of all by inanition. It nevertheless seems to me to be unquestionable that the duration of life depends on the character of metabolism, in other words, that natural death is a function of metabolism. The experiments performed by Kellogg and Bell ('04 *b*), by Pictet ('04) and by Northrop ('17) on larvæ as well as those by Loeb and Northrop ('17) on fully-developed insects of Drosophila show that by means of inadequate food we may elicit considerable changes of the duration of development or of life of these organisms. As investigations on the hunger metabolism evidence distinct differences of the digested substances and of the character of their disintegration in comparison to normal metabolism, it ought to be inferred that, if death is a function of metabolism, insufficient feeding may also influence the moment of natural death. Schultz ('05) emphasizes that certain animals undergoing periods of hibernal (or æstival) sleep which is connected with very restricted metabolism live very long in comparison with those having no such periods of rest. Stress must be laid on the fact that in my experiments moths deriving from starved caterpillars in which the development was much prolonged or

delayed are much older organisms than the control moths. As the imago of *Lymantria dispar* L. never takes any food, the quantity of provisions stored in its body decides the duration of this life-period. From histological research it follows that the amount of this provision which may be noticed in the imago (the so-called adipose body) is in the starved specimens, in relation to their decreased body, not at all smaller than in the controls. This is due to the fact that the starved caterpillars do not form normally sized chrysalids, which contain smaller quantities of the mentioned body, but they are transformed into smaller, even dwarfed specimens. By this circumstance it might be explained why both categories of moths live in general equally long. But in the case of the "starved" moths derived from caterpillars the life of which has undergone a very remarkable prolongation it is obvious that the hunger metabolism during development caused retardation of natural death of the organism. This I consider to point clearly to the conception of death as a function of the character of general metabolism. According to Ruzicka's researches ('17), the newts, when totally deprived of food, undergo moults more rapidly than control specimens. In connection with my experiments it would be very interesting to ascertain whether the duration of life of such newts undergoes changes.

More detailed considerations on the cause of especially favorable influence of intermittent starvation on duration of life of animals are to be found in my former papers (Kopeć, '22 *a* and *b*.)

5. Summary.

1. Intermittent starvation of young caterpillars of *Lymantria dispar* L. causes considerable prolongation of the larval life as well as a certain abbreviation of the pupal period but has no influence on the duration of life of the imago. These changes increase in proportion as more intense starvation is applied. Larger effects are elicited by longer and less frequent than by more frequent, but shorter, feeding intervals.

2. The differences of results obtained by various authors in regard to the influence of starvation on metamorphosis depend on differences of age of the animals experimented upon. The caterpillars subjected to inanition approximately from the seventh

day after their last moult had retarded pupation, whereas this process is accelerated by starvation of animals approximately since the tenth day after the last moult.

3. The development of imaginal discs is not the consequence of histolytical processes which cause pupation of caterpillars, but they take place simultaneously from the first days of larval life. The brain causes, probably by its secretion (or secretions), histolysis of larval tissues and it also seems to check in the caterpillar the development of the imaginal discs.

4. The prolongation of the larval period in starved specimens may be explained by certain disturbances in the hypothetical secretory function of the larval brain, which are caused by inanition; the abbreviation of the pupal stage may be ascribed to analogous decrease of the influence of this organ, which retards the development of imaginal discs.

5. The average limit of larval growth expressed in the average weight of the new-formed chrysalids is in direct proportion to the quantity of food given and inverse to the prolongation of larval and to the abbreviation of pupal life. (The decrease of weight of caterpillars is larger in cases of longer though more rare food-intervals than in cases of more frequent but shorter ones.) These rules may be applied only to differently starved whole experimental materials, having no application to separate specimens of separate broods, both starved and control.

6. The capacity to grow as well as the capacity to undergo metamorphosis exists in starved specimens far beyond the age at which control caterpillars cease to grow and undergo transformation.

7. During long lasting starvation organisms get accustomed to the abnormal conditions: the rate of growth of the caterpillars starved every second day during their whole life becomes in time considerably greater than that of specimens analogically deprived of food since their last moult but one.

8. Hunger death of caterpillars is probably caused first of all by exhaustion of reserve substances. Natural death of the imago probably is a function of the character of metabolism, as death is delayed by the changed metabolism of intermittent starvation.

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ON THE HETEROGENEOUS INFLUENCE OF STARVATION OF MALE AND OF FEMALE INSECTS ON THEIR OFFSPRING.

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(WITH 4 TABLES.)

Our knowledge of the influence exerted by insufficient feeding of the parents on their offspring is almost entirely confined to certain observations made on fetuses of the Mammalia. It has been noticed that these fetuses are able to take from tissues of the mother the substances which are more or less lacking in her food. The problem as to whether and to what degree starvation of either of the parents separately has any influence on the total development of their progeny from birth till sexual maturity has so far as I know, not as yet been methodically investigated.

Kellogg and Bell ('04) ascertained that starved silkworms mated with each other produce caterpillars which develop more slowly and produce lighter moths, in spite of the normal feeding conditions of this generation. But, as the authors have exclusively mated the starved specimens with each other they have not discriminated the influence of starvation of either of the parents separately. For this discrimination it was necessary to mate both females subjected to starvation with control males and normal females with starved males. These two experiments were undertaken in the present research.

The experiments were performed on the moth *Lymantria dispar* L. The whole material came from series *D* of my investigations on the influence of starvation of caterpillars on the development of insects, (Kopeć, '21 and '23). As *Lymantria dispar* does not take any food in its imaginal stage, starvation of the parents can take place only during their larval stage. In that series of experiments the caterpillars were fed every second day since their last moult but one. Adult moths which develop from these

[1] Paper from the laboratory of Experimental Morphology. Cf. *Mém. de l'Institut Nat. Polonais d'Économie Rurale à Pulawy*, Vol. 2, 1922.

caterpillars were mated to control moths which were at my disposal. In the filial generation the conditions of rearing were identical in each lot. Food was given daily and in superfluity. As all lots originated from one pair of moths caught in 1917, the material is to a certain degree genetically homogeneous.

The following physiological characters of the filial generation were taken into account: (1) The duration of the larval life, which in the parental generation had undergone considerable prolongation owing to inanition, (2) the duration of the pupal stage which in the parents had undergone a certain abbreviation, (3) the limits of growth of the caterpillars expressed by the weight of fresh pupæ which in the starved parental generation were much smaller than control specimens. Apart from this, the size of the eggs out of which the first filial generation developed, their number and their capacity to develop as well as the mortality of caterpillars and of chrysalids have been examined. The changes of the above-mentioned characters were in the filial generation in part much smaller than in the parents. I therefore did not use for my calculations in these experiments the averages of a character for each lot separately, but in each experiment I joined all specimens of either sex of all lots into class-frequencies as usual and I calculated the average values for all specimens together with the probable error of these averages ($A \pm E_A$). On comparing any character of the control and of the experimental material only such differences are considered as essential, *i.e.*, biometrically well grounded, which are larger than four times their probable error $\left(\frac{\text{Diff.}}{E_{\text{Diff.}}} > 4\right)$. When the ratio $\frac{\text{Diff.}}{E_{\text{Diff.}}}$ is < 4 the differences are considered as biometrically not essential and the character as not changed.

The Offspring of Females Subjected to Starvation Mated with Control Males.

From Table I. we may infer that the number of eggs laid by these specimens was much smaller than that produced by control females mated with control males. On the other hand, the size of the eggs was biometrically identical in both cases, as is evidenced in Table II. Almost all eggs from starved females were capable of development, although a large number of the develop-

TABLE I.

	Offspring of Control Females Mated with Control Males.				Offspring of Starved Females Mated with Control Males.				Offspring of Control Females Mated with Starved Males.			
	Number of Eggs.	Per Cent. of Eggs from which Caterpillars Hatched.	Per Cent. Caterpillars which Under-went Pupation.	Per Cent. Chrysalids from which Moths Emerged.	Number of Eggs.	Per Cent. of Eggs from which Caterpillars Hatched.	Per Cent. Caterpillars which Under-went Pupation.	Per Cent. Chrysalids from which Moths Emerged.	Number of Eggs.	Per Cent. of Eggs from which Caterpillars Hatched.	Per Cent. Caterpillars which Under-went Pupation.	Per Cent. Chrysalids from which Moths. Emerged.
In separate lots	197	48.7	20.8	100.0	69	31.8	31.8	100.0	346	90.7	10.1	68.7
	346	61.2	29.7	74.6	107	53.2	29.8	94.1	453	94.7	0.9	75.0
	295	52.5	52.2	93.8	268	46.9	5.6	85.7	377	83.2	2.5	75.0
	348	42.5	27.0	85.0	262	51.1	32.0	75.0	335	86.2	12.1	65.7
	160	75.0	27.5	87.8	177	45.1	32.5	88.4	376	78.4	4.0	75.0
	242	82.6	5.0	90.0	80	25.0	35.0	71.4	319	25.0	2.5	50.0
	277	94.6	28.6	86.6	99	90.9	55.5	76.0	411	90.9	15.5	70.5
	381	50.1	27.7	92.4	129	68.9	20.2	88.8				
					222	89.6	25.1	84.0				
					49	51.0	24.0	83.3				
					97	82.4	68.0	98.0				
					102	78.4	62.5	96.0				
					57	24.5	50.0	71.4				
Average..	280.7	63.4	27.3	88.7	132.1	56.8	36.3	85.5	373.8	78.4	6.8	68.5

ing eggs died in time. (The number of eggs which did not develop at all was not larger than 1 per cent. of all eggs in the control material as well as in that experimented upon.) The percentage of eggs from which caterpillars hatched was approximately identical in this experimental material and in the control specimens. The same may be said as to the percentage of caterpillars which

TABLE II.

A—average; E_A—probable error of the average; σ—standard deviation; n—number of specimens; Diff.—difference between the control and the experimental material; $E_{Diff.}$—probable error of this difference.

DATA CONCERNING MAXIMAL DIAMETERS OF EGGS, IN μ.
DIFFERENCES OF THE ABOVE AVERAGES, IN μ.

Materials.	$A \pm E_A$.	σ.	n.
Eggs of control females mated with control males	1234.65±2.319	53.81	245
Eggs of starved females mated with control males	1237.09±1.783	51.61	381
Eggs of control females mated with starved males	1238.00±2.064	44.35	210

Averages Compared.	Diff. $\pm E_{Diff.}$	$\frac{Diff.}{E_{Diff.}}$	Nature of Difference.
Difference between the average size of control eggs and of that of the eggs from starved females mated with control males.	−2.44±2.925	0.83	Not essential.
Difference between the average size of control eggs and of that of the eggs from control females mated with starved males.	−3.35±3.104	1.08	Not essential.

underwent pupation and of the chrysalids which developed into adult moths (cf. Table I.). Pupation of caterpillars and hatching of moths having been checked once daily during one and the same hour, the limits of this life-period were determined with exactness up to 24 hours. The data of Tables III. and IV. referring to the duration of the larval and of the pupal life in the progeny of starved females mated with control males as well as the same data of the control material (control ♀ ♀ × control ♂ ♂) show that the duration of the larval life is not essentially changed but the duration of the pupal period of the offspring of the females subjected to starvation has undergone essential abbreviation. The limits of larval growth have been determined, as in the

experiments with the parental generation, by the weight of new-formed chrysalids. As pupation was checked once a day it ought to be emphazised that during the first 24 hours the chrysalid did not lose more than 0.15 to 0.28 per cent. of the original weight of the chrysalid immediately after its emergence from the larval skin. If we take into consideration the appertaining strict biometrical data of Tables III. and IV., as well as the ratio $\frac{\text{Diff.}}{E_{\text{Diff.}}}$, we may ascertain that the weight of the chrysalids underwent no

TABLE III.

DATA REFERRING TO THE DURATION OF LARVAL AND PUPAL LIFE AND TO THE WEIGHT OF CHRYSALIDS.

A—average; E_A—probable error of the average; σ—standard deviation; n—number of specimens.

	Materials.	Sex of the Specimens.	$A \pm E_A$.	σ.	n.
Duration of larval life in days.	Caterpillars from control females mated with control males.	♂♂	52.81 ± 0.247	4.42	146
		♀♀	63.21 ± 0.309	6.93	229
	Caterpillars from starved females mated with control males.	♂♂	51.66 ± 0.217	4.23	173
		♀♀	63.73 ± 0.320	6.14	167
	Caterpillars from control females mated with starved males.	♂♂	52.22 ± 0.325	4.57	90
		♀♀	63.39 ± 0.762	9.52	71
Duration of pupal life in days.	Chrysalids from control females mated with control males.	♂♂	22.83 ± 0.0568	0.93	122
		♀♀	19.15 ± 0.0525	1.12	207
	Chrysalids from starved females mated with control males.	♂♂	22.21 ± 0.0521	0.93	145
		♀♀	18.51 ± 0.0420	0.76	149
	Chrysalids from control females mated with starved males.	♂♂	21.91 ± 0.0771	0.84	54
		♀♀	18.45 ± 0.1275	1.44	58
Weight of chrysalids in mg.	Chrysalids from control females mated with control males.	♂♂	283.56 ± 4.63	82.92	146
		♀♀	758.51 ± 13.04	292.64	229
	Chrysalids from starved females mated with control males.	♂♂	303.76 ± 4.17	81.38	173
		♀♀	794.61 ± 18.02	345.25	167
	Chrysalids from control females mated with starved males.	♂♂	216.67 ± 4.14	58.21	90
		♀♀	609.86 ± 19.34	241.65	71

TABLE IV.

DIFFERENCES OF AVERAGE DURATION OF LARVAL AND OF PUPAL LIFE AS WELL AS OF WEIGHT OF CHRYSALIDS BETWEEN THE CONTROL AND THE EXPERIMENTAL MATERIAL.

Diff.—difference between the control and the experimental material. $E_{Diff.}$—probable error of this difference.

	Averages Compared.	Sex of the Specimens.	Diff. ± $E_{Diff.}$	$\frac{Diff.}{E_{Diff.}}$	Nature of Difference.
Data concerning larval life, in days.	Difference between the average duration of larval life of control specimens and of that of specimens deriving from starved females mated with control males.	♂♂	1.15± 0.329	3.49	Not essential.
		♀♀	− 0.52± 0.445	1.17	"
	Difference between the average duration of larval life of control specimens and of that of specimens deriving from control females mated with starved males.	♂♂	0.59± 0.408	1.44	Not essential.
		♀♀	− 0.18± 0.822	0.22	"
Data concerning pupal life, in days.	Difference between the average duration of pupal life of control specimens and of that of specimens deriving from starved females mated with control males.	♂♂	0.62± 0.0771	8.04	Essential.
		♀♀	0.64± 0.0672	9.52	"
	Difference between the average duration of pupal life of control specimens and of that of specimens deriving from control females mated with starved males.	♂♂	0.92± 0.0958	9.60	Essential.
		♀♀	0.70± 0.1378	5.08	"
Data concerning weight of chrysalids, in mg.	Difference between the average weight of control pupæ and of that of pupæ deriving from starved females mated with control males.	♂♂	−20.20± 6.23	3.24	Not essential.
		♀♀	−36.10±22.24	1.62	"
	Difference between the average weight of control pupæ and of that of pupæ deriving from control females mated with starved males.	♂♂	66.89± 6.21	10.77	Essential.
		♀♀	148.65±23.32	6.37	"

essential change in this experimental material. (The pupæ are here even heavier, but the differences are smaller than their fourfold error.)

The Offspring of Control Females Mated with Males Subjected to Starvation.

As the control females mated with starved males were by chance mostly larger than the control females mated with control males, they laid somewhat larger quantities of eggs (Table I.). The egg size, however, was identical in this material and in that of the control, as may clearly be inferred from Table II. Also in this experiment the percentage of eggs which did not develop at all was not larger than 1 per cent. of all eggs.

An exact inquiry into the data of Table I. shows that the capacity of the spermatozoa from starved males to fertilize and to timulate eggs to total development is, if not larger, at least nots smaller than in spermatozoa of control specimens. On the other hand, mortality of caterpillars as well as of chrysalids in this experiment was undoubtedly larger than in control materials. From data of Tables III. and IV. we see that the larval life in the offspring of control females mated with males subjected to inanition does not undergo any essential change. On the other hand, the duration of the pupal stage is essentially shortened in this experiment. On comparing the data of the same tables it is also found that in contrast to the offspring of starved females mated with control males, the offspring of males subjected to starvation and of control females is lighter than the progeny of control material.

General Conclusions and Summary.

The above experiments are the first to discriminate strictly the influence of starvation exerted on the offspring by either of the parents separately. The starved females the progeny of which was used for this research were much smaller than the control specimens, their limit of growth being only 48.4 per cent. of the normal limit of growth (Kopeć, '21 and '23, Table III.). Nevertheless, the body-weight of the offspring of such starved females was at least normal. This leads to the supposition that in females hunger metabolism does not bring about any quantitatively

unfavorable changes in the reserve substances out of which eggs are developed in the chrysalids. On the other hand, we have to do here with a special regulation of quantitative relations, consisting of two factors: the number of eggs laid by the females undergoes in general considerable reduction, but the egg size does not exhibit any changes, which points to unchanged quantity of food substances for the developing embryo in each "starved" egg.

The normal size of eggs layed by starved females together with the decreased number of the eggs speak in favor of the supposition that in case of insufficient feeding of an animal the food substances may be exclusively used up by a certain number of eggs which develop better than the remaining, owing to their arrangement in the ovary or to other conditions. This is caused by the competition between separate elements, which undoubtedly takes place in the starved ovary analogous to the struggle between individuals of a brood, which develops in unfavorable conditions. Owing to this rivalry, only part of the eggs undergo development, directly or indirectly at the expense of neighboring elements, as the number of eggs contained in "starved" chrysalids is considerably larger than the number of fully developed eggs in the mature females. Notwithstanding the capacity of certain "starved" eggs to develop at the expense of other eggs, this average size does not exceed essentially the normal average limit, which points to the dependence of egg size on certain internal factors by which the limits of the growth of eggs are determined. These factors are undoubtedly synonymous with "genes" which determine in genetical sense the size of eggs independently of the size of the females by which the eggs are produced. In spite of this evolutive independence during the development of a fetus or of an egg, there probably exists a certain limit of degree and of duration of starvation which may be applied to a female, beyond which limit the progeny does not attain its normal size. The assumption that this limit may easily be transgressed is supported by the fact that certain small unfavorable changes in feeding birds cause a considerable diminution of their eggs and even check the further processes of development of the sexual elements. This limit was undoubtedly transgressed in the experiments made by Woltereck ('08–'11,) in which the

offspring parthenogenetically produced by starved *Daphniæ* was smaller than control specimens.

In contrast to the offspring of starved females the progeny of similarly treated males were essentially lighter than those produced by control males. The unfavorable influence of starvation of males was evidenced by increased mortality of caterpillars as well as of chrysalids, which could not be observed in the offspring of starved females. As egg size was also in this experiment normal, the cause of these changes ought to be ascribed to the specific influence exerted by starvation of males on their spermatozoa.

A great influence on the offspring of the Mammalia exerted by certain substances applied to their fathers was demonstrated, *e.g.*, by experiments on the influence of alcohol made by Stockard and his collaborators ('12– '18) and others, by the experiments on lead performed by Cole and Bachhuber ('14) and by those of Guyer and Smith ('18 and '20) on a special lens dissolving serum. These researches have shown a considerable susceptibility of the spermatozoa to certain extrinsic specific substances. My experiments prove that normal development of the spermatozoa may also be influenced by certain natural changes of metabolism caused by inanition. Histological research has shown that the males which develop from starved caterpillars contain the same quantities of reserve substances relatively to their diminished size as control specimens; as the testicles of the moth which develop in the caterpillar at the expense of these substances are too small organs to play an important rôle in the nutritive balance of the chrysalis undergoing metamorphosis, it ought to be supposed that the influence exerted on the spermatozoa by hunger metabolism consists in qualitative and not in quantitative changes of the chemical constitution of the spermatozoa. In my experiments I did not succeed in ascertaining any marked histological changes in the developing "starved" testicle, neither as to the evolutive rate, nor as to the structure of the sexual elements. Physiological dimorphism of metabolism in the two sexes is certainly the cause why starvation of females does not elicit analogous unfavorable quantitative changes in the egg.

From the above results we may infer that the decreased weight of the offspring of two starved parents determined in silkworms

by Kellogg and Bell ('04) was exclusively due to the influence of the spermatozoa from the starved males, unless the limits beyond which we ought to expect a noxious influence of the starvation of females on their progeny had been transgressed, owing to different degree of starvation. It ought to be mentioned that in certain species the development of the sexual elements occurs in natural conditions during periods of total physiological starvation. Miescher ('97) ascertained that in the salmon which does not take any food during the development of its sexual glands its very large ovaries and testicles grow at the expense of muscles. This fact, however, is in no discrepancy with my results as it may be that certain special feeding experiments with salmon—if they are practically possible—would, as in moths, elicit in this fish changes as to the size of the offspring which in natural conditions develops here from "starved" spermatozoa. The generalization of my results obtained in insects to other animals, especially to vertebrates, would be premature. I nevertheless believe that my present contribution will have a certain general interest in regard to the great biological importance of the discussed problem.

The duration of the larval period does not undergo any essential change in the offspring of starved females nor in that of the starved males either. The pupal stage, on the contrary, is essentially changed in both cases. My conception of the physiology of insect metamorphosis is supported by such behavior of the progeny. According to my opinion the larval brain elicits by its secretion or secretions the histolysis of larval tissues, which puts an end to larval life (Kopeč, '17 and '22). It also checks in the larva the development of the embryonal discs from which the organs of the imago are finally formed in the chrysalids ('21 and '23). The prolongation of the larval life and the simultaneous abbreviation of the pupal stage caused by starvation of caterpillars has been interpreted as due to delayed or decreased secretoric function of the brain. If the prolongation of larval life elicited by starvation of the mother or of the father is due to delayed or decreased production of the substance by which histolysis of tissues is brought about, no wonder that in the filial generation there is no change in respect to the duration of larval life: in these experiments the larval organism of the filial generation remains unchanged as to its preparation for histolysis. The

duration of the pupal stage in the filial generation must be on the contrary explained in a different manner. It has been shown that the abbreviation of the pupal period and consequently the acceleration of development of the embryonal discs was brought about even by temporary starvation of very young caterpillars, viz., lasting 20 days after hatching from eggs, *i.e.*, at a time when there are in the larval organism not yet any traces of histolytical processes characteristic of pupation (Kopeć, '21 and '23). It may therefore easily be assumed that not only the embryonal discs of the animal subjected to inanition, but also the evolutive factors contained in their sexual cells, which cause the evolution of the embryonal discs in the filial generation, are stimulated to development by starvation and by the decreased checking function of the brain connected therewith. In the caterpillars of this filial generation the brain finds therefore the embryonal discs which are more physiologically advanced and this fact eventually leads to a certain abbreviation of the pupal stage of the progeny of starved females or of starved males.

On surveying the results of the foregoing inquiry the following summary may be given:

1. The females of the moth *Lymantria dispar* L. derived from starved caterpillars mated with control males laid smaller quantities of eggs than control specimens, but the dimensions of the eggs and the weight of the pupæ developing from them did not undergo any essential changes. The capacity of the egg to develop and the mortality of caterpillars as well as of chrysalids are not changed by the starvation applied in these experiments as compared with control material.

2. The morphological structure of the spermatozoa of males deriving from similarly starved caterpillars is unchanged and they have the normal capacity for stimulating eggs to development, but the mortality of the resulting caterpillars and chrysalids is distinctly larger than that of the control material. The weight of the pupæ in the progeny of starved males undergoes essential decrease.

3. Distinctly injurious effects were consequently brought about in the offspring by inanition of the males, in contrast to inanition of the females. The causes of the different behavior of either of the starved parents ought to be referred to metabolism probably

different in the two sexes. Changes elicited in the structure of the spermatozoa by starvation of males are most probably qualitative.

4. The prolongation of larval life which had been noticed in the starved male as well as female caterpillars could not be ascertained in their offspring. The pupal stage, on the contrary, which had been much shortened in starved specimens underwent in the offspring of starved females as well as in the offspring of starved males an analogous change which leads to acceleration of metamorphosis. Such behavior of the progeny of starved specimens supports the author's former opinion that insect metamorphosis is checked by the secretoric function of the larval brain.

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A NEW FIELD METHOD OF INVESTIGATING THE HYDROTROPISMS OF FRESH-WATER INVERTEBRATES.

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INTRODUCTION.

During the past quarter of a century the tropism idea has stimulated intensive investigation of the factors that serve as directive forces in the behavior of animals. Gravitation, light, heat, contact, wind and water currents, as factors of orientation, have been much investigated; but, the directive influence of bodies of water as such has received scant attention. In her learned textbook,[3] Professor Washburn does not discuss the subject. In his book on animal intelligence [2] Professor Holmes devotes a whole chapter to tropisms. He discusses; chemotaxis, geotaxis, thigmotaxis, rheotaxis, phototaxis, thermotaxis and electrotaxis; but, not a word about hydrotaxis. Professor Bouvier, in a recent work,[1] says more on this topic than I have seen in any other book on animal behavior. He writes:

"No less than heat, water is necessary to living beings, for it constitutes the greater portion of their protoplasm and plays a part in almost all their internal changes. Also, all organisms are sensitive to variations of humidity in the space surrounding them, and with a great number this sensitiveness takes the form of a directive orientation which is called hydrotropism.

"We know the famous experiments made by Stahl, in 1884, on the hydrotropism of the fungi of the genus *Æthalium* (tanning fungus). The plasmodial mass of these plants, during the vegetation period, enters the tan, making for the humidity necessary

1. *Note by the editor.* This paper was received for publication January 28, 1923. The author died February 14, 1923. Dr. Turner was a student of Professor C. O. Whitman, and received the degree of Doctor of Philosophy from the University of Chicago in 1907. His thesis was on "The Homing of Ants: An Experimental Study of Ant Behavior." He continued his patient, thorough studies of animal behavior to the time of his death, as the present contribution will testify. Several studies by Dr. Turner have appeared in the pages of the BIOLOGICAL BULLETIN.

to it, and remounts to the surface in a dry *milieu* when it is going to form its spores which serve its multiplication. Its hydrotropism is positive. This is the case with the beetles of the genera *Haliplus* and *Hydroporus*. Wheeler (1899) had taken from a pool a tuft of aquatic plants where these insects swarm. He says: 'As soon as the beetles could come out and disengage themselves from the plant they turned, with a common accord, toward the sea and to it directed their steps. As this was a distance of about twenty feet, the little creatures could not see the water, and I was led to believe that they had some means of perceiving a source of moisture and acted accordingly.

"Aquatic bugs act the same way when they are taken from the place in which they live, and we know that the land crabs go a long distance to water when they are ready to place their progeny. The proper degree of humidity differs, moreover, with different species. Wheeler reports that *Bembidium*, *Elaphrus*, *Omophron*, and other small Coleoptera which bury themselves in the sandy beaches, leave their burrows and come out into the open air when one throws a little water on their strand. This is negative hydrotropism. It is well known to collecting entomologists, who use it in making captures."

Weiss [4] reports two cases of what he calls positive hydrotropism. When specimens of the wingless *Gerris marginatus* were removed one to nine yards from the pond they immediately returned to it. When removed ten yards from the water they had some trouble in getting started in the right direction; but finally reached the pond. Thirty yards from the water they seemed to be lost. The case of *Dineutes assimilis*, a winged beetle, is even more interesting. When removed nine or ten feet from the pond it tried to walk to the pond, then arose and flew directly to it. When removed to a distance of seventy-five feet, it walked about in all directions, then arose, on its wings, to a height of twenty feet and flew directly to the water. When removed half a mile from the pond, it soared in a widening sub-spiral to a height of seventy-five feet and then flew off in the direction of the water. He does not know whether it reached the water or not.

It is not claimed that the above resumé contains all that has been written on this topic. It is quite likely that some articles have been overlooked. However, the fact that a diligent search

has supplied only this suggests that very little attention has been paid to the topic. This may be because no simple means of investigating it has been published. The purpose of this paper is to supply that lack and to apply the method to the study of a few forms selected from different strata of the water. As types of forms that creep along the bottom of the water or along vegetation but do not leave the water, I selected a snail [*Planorbis* (*Helisoma*) *antrosum* Con.] and a dragon-fly nymph. As examples of forms that spend most of their time on the bottom, but which, at times, leave the water, I have slected the crayfish [*Cambarus* (*Faxonius*) *propinquus* Girard] and a scavenger beetle (*Tropisternus* sp. ?). As types of forms that live on the surface of the water, I have selected the giant water strider (*Gerris remigis* Say), which, except during the hibernating season, is practically a permanent inhabitant of the film, and the whirligig beetle (*Gyrinus* sp.?) which occasionally dives and which migrates from pond to pond.

Technique.

To be able to study the movements of animals with a certainty that the movements were not influenced by either gravitation, the sun's rays, chemical stimuli or contact stimuli, I devised what may be appropriately called a checker-board plate. This consists of a board twenty inches wide and twenty-four inches long which is covered with white oilcloth that has been subdivided, by printed lines, into one inch squares (Fig. 1). The four edges of the board were bisected by straight lines which crossed, at right-angles at the center of the board. Diagonal lines, bisecting these central right angles extended outward to the edges of the board. The tips of the four lines mentioned were named; 0 degrees, 45 degrees, 90 degrees, 135 degrees, 180 degrees, 225 degrees, 270 degrees, 315 degrees. By means of a level and leveling devices this board was made perfectly horizontal. Being horizontal, gravitation could not influence the movements. Being an absolutely smooth surface, the movements could not be influenced by contact stimuli. Being covered with oil-cloth, it was possible, by washing, to keep it free from chemical stimuli. It was protected from the sun by means of a screen of some sort; this made it impossible for the sun's rays to influence

the movements. The checker-board pattern made it easy to watch the movements of the animals.

Using a scale of one-eighth of an inch equals an inch sheets of cross section paper were cut to resemble the board, and the tips of radii labeled in the same manner as on the board. These were carried to the field in a loose-leaf cover.

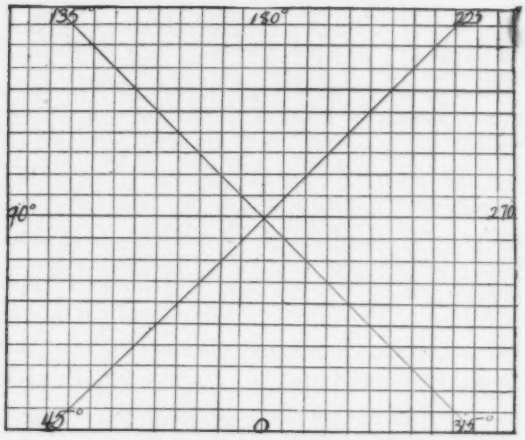

FIG. 1. Checkerboard Plate.

At the beginning of a series of experiments the board is placed on the ground and adjusted until it is perfectly horizontal. If necessary, it is screened from the sun. One of the sheets is then numbered to correspond with the specimen to be investigated. On the reverse side is recorded the position of the sun, the position of the water and the direction in which the animal's head is to be placed. In describing these positions the labeled tips of the radii are used as reference points. The animal is then placed in the center of the board with its head in the desired direction and its movements traced on the cross-section paper. When the animal reaches the edge of the board, it is returned to its original position. This is repeated until it has reached the edge of the board five times. It is then replaced in the water a short time and then returned to the center of the board with its head facing a new position. It is allowed to make five trips in that case; these are recorded on a new sheet. This

is repeated over and over again, using one sheet of paper for each five experiments, until the creature has worked with its head facing all eight of the radii tips. This gives forty experiments and is called a series. Work is continued until one has secured records of as many series as is thought necessary. In the work recorded in this paper an attempt was made to secure between twelve and thirteen series with each species investigated.

In practice it is not always possible to secure a complete series from an individual. Sometimes the individual would escape before the series was completed, at others fatigue would put an end to the experiments at an early stage. In some cases it was necessary to return the specimen to the water at the close of each experiment, instead of at the close of each five. Two investigators can do more effective work than one; one giving his entire time to looking after the animal and the other recording what happens. This is especially important when dealing with forms that fly and with crawling forms that move rapidly. After a little experience one recognizes the movements preliminary to flight. Usually a small glass cover placed over the specimen for a moment will check the attempt to fly.

In the work performed in connection with this article, on returning from the field the results of the days work were recorded on tables with the following captions;

TABLE I.

Name of Species.	Number of Specimen.	Position of Sun.	Position of Water.	Head Pointed towards.	Number of Trials.	Nature of the Water.	Time of Day.	Final Movement.						Sex.	Age.
								Toward Water.	Not toward Water.	Toward Sun.	Not toward Sun.	Way Head Pointed.	Not Way Head Pointed.		

Later these tables were condensed into those published in this article.

In the experiments recorded in this article the side of the

board marked O, always faced the water. If there had been an unlimited number of experiments, twenty-five per cent of movements toward the water would indicate that the movements were random and not influenced by the position of the water. Since the number of experiments was limited to about 500, unless the movements toward water were much more than twenty five per cent. the movements were considered random. In recording movements toward the position of the sun and the initial position of the insect's head, eight possible directions were considered. In each of these cases, if there had been an infinite series of experiments, 12.5 per cent. of movements in a certain direction would indicate random movements. Hence, since there were only about 500 experiments, unless the per cent of movements in a certain direction was much more than 12.5 the movements were considered random.

With the animals investigated in this connection, in addition to the experiments with the checker-board plate, the creatures were set free, at definite distances from the water, and their movements watched. If the experiments demonstrated a tendency to move toward the water, then the specimens were blinded and the experiments repeated.

Dragon-fly Nymphs (Plate I).

In experimenting with the checker-board plate fourteen individuals were used. In some cases the board was ten feet from the water; in others it was fifteen. Five hundred and fifty-six experiments were performed. The results are recorded in Table II.

In an infinite series of experiments, if the factors considered did not influence the movements in any manner, 25 per cent. of the movements should be toward water, 12.5 per cent. toward the position of the sun and 12.5 per cent. to the way the head originally pointed. The actual results were 25 per cent toward water, 20.5 per cent. toward the position of the sun and 23.3 per cent. toward the way the head originally pointed. Only three individuals made more than thirty per cent. of movements to water. Evidently, dragon-fly nymphs, when moving about on the checker-board plate are not influence by the nearness of bodies of water.

TABLE II.

DRAGON-FLY NYMPHS.

Specimen Number.	Number of Trials.	Kind of Water.	Initial Positions.	Per Cent. of Final Movements.						Time of Day.
				Toward Water.	Not toward Water.	Toward Sun.	Not toward Sun.	Way Head Pointed.	Not Way Head Pointed.	
1	10	Pond	2	10	90	10	90	30	70	A.M.
2	3	Pond	1	0	100	100	0	0	100	A.M.
3	5	Lake	1	100	0	0	100	0	100	A.M.
4	18	Pond	4	29	71	11	89	33	67	P.M.
5	43	Lake	8	30	70	21	79	30	70	A.M.
6	5	Pond	1	0	100	0	100	40	60	P.M.
7	80	Pond	8	46	54	37	63	21	79	P.M.
8	40	Pond	8	7	93	0	100	25	75	P.M.
9	80	Pond	8	17	83	15	85	28	72	Both
10	40	Pond	8	22	78	5	95	15	85	A.M.
11	167	Pond	8	57	43	0	100	11	89	Both
12	10	Pond	2	10	90	20	80	20	80	A.M.
13	25	Pond	5	12	88	48	52	24	76	P.M.
14	30	Pond	4	10	90	20	80	50	50	A.M.
Total	556	Av.-	4.8	25	75	20.5	79.5	23.3	76.7	

NORMAL CRAYFISH [*Cambarus* (*Faxonius*) *propinquus* GIRARD] (PLATE II).

In experimenting with the checker-board plate fourteen individuals were used,varying in length from one and a half inches to three inches. In most cases the board was placed within five or ten feet of the water, in some cases it was forty feet from the water and, in a very few cases, it was fifty feet away. In most cases the experiments were conducted near the river or brook from which the crayfish were obtained. In others the crayfish were carried to the borders of some lake or pond. The results are recorded in Table III.

In an infinite series of experiments twenty-five per cent. of movements toward the water is what should be expected if the water has not directive influence on the individuals. The experiments yielded 67.5 of movements toward the water. Three individuals made 100 per cent. of movements toward the water, four between 90 and 100 per cent. In only four cases did the per cent. fall under 50 and the lowest of those was 36. Evidently

this crayfish, when moving on the checkerboard plate, is largely directed by the nearest body of water. It acts as though it were positively hydrotropic.

TABLE III.

THE CRAYFISH [*Cambarus* (*Faxonius*) *propinquus* Girard].

Specimen Number.	Number of Trials.	Size.	Kind of Water.	Sex.	Initial Positions.	Per Cent. of Final Movements.						Time of Day.
						Toward Water.	Not Toward Water.	Toward Sun.	Not Toward Sun.	Way Head Pointed.	Not Way Head Pointed.	
1	13	1¼ in.	Brook	fem.	6	38	62	15	85	23	77	A.M.
2	7	1 in.	Brook	fem.	2	100	0	0	100	0	100	A.M.
3	3	1 in.	Brook	fem.	1	100	0	0	100	0	100	A.M.
4	80	2 in.	Pond	male	8	41	59	1	99	11	89	Both
5	40	1 in.	Pond	fem.	8	97	3	0	100	0	100	P.M.
6	40	1 in.	Lake	fem.	8	75	25	30	70	22	78	P.M.
7	40	1 in.	Lake	fem.	8	100	0	2	98	15	85	P.M.
8	40	1¼ in.	Lake	fem.	8	65	35	13	87	8	92	P.M.
9	40	3 in.	Pond	male	8	52	48	0	100	28	72	P.M.
10	80	1½ in.	Pond	fem.	8	91	9	3	97	23	77	A.M.
11	7	1½ in.	Pond	fem.	2	57	43	0	100	14	86	A.M.
12	80	2½ in.	River	fem.	8	56	44	7	93	35	65	Both
13	45	1 in.	River	male	8	36	64	11	89	29	71	A.M.
14	40	1 in.	River	male	8	38	62	15	85	55	45	A.M.
Total	535	Average.....			6.5	67.5	32.5	6.9	93.1	18.8	81.2	

In an infinite series of experiments, 12.5 per cent. of movements toward the position of the sun would indicate that brightness of the surrounding region (the direct rays of the sun did not have access to the specimens) did not have a directive effect. The experiments yielded only 6.7 per cent. of movements toward the position of the sun. Four individuals made zero per cent. of movements toward the position of the sun, three less than four per cent., in only four cases did the per cent. become more than twelve. Evidently, on the checkerboard plate, the crayfish shows a tendency to move away from the brightest part of the field.

In an infinite series of experiments 12.5 per cent. of movements in the direction the head originally pointed would indicate that the position of the head had not influenced the movements.

These experiments yielded 18.8 per cent. of movements in the direction the head originally pointed. This is so near to what theory demands that it is evident that the original position of the head has not appreciably influenced the final movements of the individual.

In the matters considered no marked difference was observed between males and females.

At the close of each series of experiments mentioned above, the crayfish was placed on the ground, with its head away from the water. The distances from the water ranged from ten to fifty feet. In each case the crayfish finally turned and went to the water, even going around obstacles.

On another occasion twelve crayfish were placed, one at a time, on the ground, within ten feet of the body of water from which they had been obtained. They were always faced away from the water. Eleven of these reached the water in a very few minutes; the twelfth roamed about for fully half an hour before it found the water. Considered alone these experiments would not permit us to draw any conclusions. The land sloped toward the water. The sun was shining. Hence gravity and the sun rays may have influenced the movements. However, the results are in harmony with the conclusions drawn from the experiments on the checkerboard plate; and, when considered in conjunction with some experiments to be described in the next section, they are illuminating.

BLINDED CRAYFISH (*Cambaraus* sp.? AND *Cambarus* (*Fraxonius*) *propinquus* Girard) (PLATE III).

The above experiments with the checkerboard plate prove conclusively that the nearest body of water, in some manner, forces the crayfish to move towards it. One naturally wonders if the eyes function in this behavior. To test the matter three types of experiments were conducted with blinded crayfish.

In the first series thirteen blinded crayfish were tested on the checkerboard plate. The eyes of five were blinded with an opaque varnish, the eyes of the other eight were amputated. The board was placed twenty feet from the water. The results are recorded in Table IV.

In an infinite series of experiments, if the factors considered did

not influence the movements, 25 per cent. of the movements should be toward water, 12.5 per cent. toward the sun and 12.5 per cent. toward the way the head was originally pointed. The experiments yielded the following results; 25.7 per cent. toward water, 15.3 per cent. toward the sun and 17.1 per cent. toward the way the head originally pointed. Evidently the movements of blind crayfish, on the checkerboard plate, are not influenced by the nearby bodies of water.

TABLE IV.

THE CRAYFISH (*Cambarus*, sp.?) BLIND (PLATE III).

Specimen Number.	Number of Trials.	Size.	Kind of Water.	Sex.	Initial Positions.	Per Cent. of Final Movements						Time of Day.
						Toward Water.	Not Toward Water.	Toward Sun.	Not Toward Sun.	Way Head Pointed.	Not Way Head Pointed.	
1	40	4 in.	Pond	fem.	8	12	88	0	100	10	90	P.M.
2	20	4 in.	Pond	male	4	40	60	25	75	5	95	P.M.
3	40	4 in.	Pond	male	8	17	83	7	93	22	78	P.M.
4	40	4 in.	Pond	fem.	8	42	58	20	80	35	65	P.M.
5	38	4 in.	Pond	male	8	45	55	11	89	21	79	P.M.
6	40	4 in.	Pond	fem.	8	25	75	15	85	15	85	P.M.
7	40	4 in.	Pond	male	8	32	68	22	78	17	83	P.M.
8	40	4 in.	Pond	male	8	20	80	18	82	18	82	P.M.
9	80	4 in.	Pond	male	8	23	77	29	71	40	60	A.M.
10	40	4 in.	Pond	fem.	8	42	58	13	87	15	85	A.M.
11	65	4 in.	Pond	fem.	8	26	74	9	91	15	85	A.M.
12	10	4 in.	Pond	male	2	10	90	30	70	10	90	A.M.
13	5	4 in.	Pond	fem.	1	0	100	0	100	0	100	A.M.
Total	498	Average.....			6.9	25.7	74.3	15.3	84.7	17.1	82.9	

These thirteen blinded crayfish were placed on the ground, within fifteen feet of a pond. Their heads were faced away from the water. The land sloped gently towards the water and the sun was shining brightly. At the end of an hour none had reached the water. Several had wandered off into the shrubbery and become lost, three had moved to within two feet of the water and then turned and walked off in another direction, some were wandering about at a greater distance from the water than their original position and three were in practically their original positions.

In another series of experiments the crayfish [*Cambarus* (*Fraxonius*) *propinquus*] were used in pairs. The eyes of one

of each pair were painted with a thick coat of lampblack mixed in liquid glue. The eyes of the other were normal. Twelve pairs were used. They were placed fifteen feet from the water. In ten cases the one with good eyes reached the water in a short time and the one with blinded eyes failed to do so. In one case neither reached the water. In another the one with blinded eyes reached the water and the one with good eyes wandered away from the water. The experiment with this last couple was repeated and yielded similar results.[1] It is probable that the eyes of this blinded crayfish were not entirely blind; because, on the next day, after the eyes had been repainted, this individual behaved like the other blinded ones. Its companion of the first day died over night; hence, it was not possible to re-test it.

These experiments with blinded crayfish, when considered in connection with the experiments with normal crayfish, demonstrate that the stimulus by means of which a body of water controls the movements of the crayfish reach the animal through the eyes.

GIANT WATER BUG [*Belostoma (Zaitha) fluminea*] (PLATE IV).

The exceptionally dry summer caused many ponds to go dry and it was impossible to secure enough specimens to conduct an extensive number of experiments. Five individuals were used and 109 experiments performed. The checkerboard plate was placed ten feet from the water. The results of these experiments are recorded in Table V.

TABLE V.

GIANT WATER BUGS [*Belostoma (Zaitha) fluminea*].

Specimen Number.	Number of Trials.	Age.	Kind of Water.	Initial Positions.	Per Cent. of Final movements.						Time of Day.
					Toward Water.	Not Toward Water.	Toward Sun.	Not Toward Sun.	Way Head Pointed.	Not Way Head Pointed.	
1	8	Adult	Pond	8	70	30	0	100	23	77	P.M.
2	10	Juv.	Pond	2	10	90	0	100	10	90	P.M.
3	40	Adult	Pond	8	50	50	5	95	30	70	P.M.
4	11	Juv.	Pond	3	73	27	0	100	36	64	P.M.
5	40	Juv.	River	8	32	68	2	98	0	100	A.M.
Total	109	Average.....		5.8	47	53	1.4	98.6	19.8	70.2	

[1] In this series of experiments two pairs were used at a time; my wife watching one pair while I watched the other.

It is probably unwise to draw conclusions from only a hundred experiments; but, these experiments suggest that the water does have a directive influence upon this giant water bug.

A Scavenger Beetle (*Tropisternus*) (Plate V).

In experimenting with the checkerboard plate nineteen individuals of this species were induced to perform 571 experiments. In most cases the board was ten to twenty feet from the water. In one case it was fifty feet (number 18); in another case it was 100 feet (number 19). The results of the experiments are recorded in Table VI.

TABLE VI.

A Scavenger Beetle (*Tropisternus*)—Adults.

Specimen Number.	Number of Trials.	Kind of Water.	Initial Positions.	Per Cent. of Final Movements.						Time of Day.
				Toward Water.	Not Toward Water.	Toward Sun.	Not Toward Sun.	Way Head Pointed.	Not Way Head Pointed.	
1	7	Lake	5	87	13	0	100	13	87	A.M.
2	2	Pond	1	50	50	0	100	0	100	A.M.
3	9	Pond	1	55	45	11	89	11	89	A.M.
4	18	Pond	3	7	93	0	100	50	50	P.M.
5	40	Pond	8	3	97	3	97	13	87	P.M.
6	40	Pond	8	53	47	53	47	20	80	P.M.
7	8	Pond	1	25	75	25	75	88	12	P.M.
8	40	Pond	8	0	100	0	100	23	77	P.M.
9	40	Pond	8	0	100	65	35	25	75	P.M.
10	40	Pond	8	35	65	0	100	8	92	P.M.
11	40	Pond	8	55	45	15	85	28	72	P.M.
12	9	Pond	2	11	89	0	100	44	56	A.M.
13	40	Pond	8	45	55	5	95	40	60	P.M.
14	10	Pond	2	10	90	0	100	40	60	A.M.
15	40	Pond	8	37	63	20	80	18	82	A.M.
16	80	Pond	8	6	94	8	92	38	62	A.M.
17	40	Pond	8	47	53	10	90	15	85	P.M.
18	40	Pond	8	45	55	0	100	20	80	P.M.
19	28	Pond	4	53	47	14	86	29	71	P.M.
Total	571	Av.-..	5.1	32.3	67.7	12	88	27	73	

In an infinite series of experiments, if the factors considered did not influence the movements, 25 per cent. should have been toward the water, 12.5 per cent. toward the sun and 12.5 per cent. toward the direction the head originally pointed. The

results obtained from the experiments were; 32.3 per cent. toward the water, 12 per cent. toward the sun, and 27 per cent. toward the direction the head originally pointed. The per cent of movements toward the water is too low to warrant the assumption that the movements of the group, as a whole, have been influenced by the water; however, certain individuals exhibit a marked tendency to move toward the water. One individual made 87 per cent. of movements toward the water; five, between 50 and 60 per cent.; three, between 40 and 50 per cent. Thus nine individuals, practically half of the individuals used in the experiments exhibited a tendency to move toward the water.

Brightness did not influence the movements. There was a slight tendency to move in the direction the head was originally pointed.

When these beetles were given their freedom, some flew and others did not. Of those that flew, more than 90 per cent. flew to the water.

Small Diving Beetle (*Laccophilus* sp.?).

To test their fitness for work on the checkerboard plate, a few experiments were conducted with a small diving beetle. On account of its proneness to fly it is a difficult subject with which to work; but, where one has sufficent patience it may be used. It might be a good idea to clip its second pair of wings. On escaping they always flew toward the water.

The Large Water Strider (*Gerris remigis* Say) (Plate VI).

In working with the checkerboard plate, 27 individuals were induced to perform 534 experiments. Seventeen individuals were used near the stream where they were captured; the remainder were taken to the shore of a lake. In most cases the board was placed from five to ten feet from the water. In a few cases it was necessary to place it twenty feet away. The results are recorded in Table VII.

In an infinite series of experiments, if the factors considered, have no directive influence on the animals, then 25 per cent. of the movements should be toward the water, 12.5 per cent. toward the sun and 12.5 per cent. toward the original position of the head. The experiments yielded 34.7 per cent. of movements

toward the water, 16 per cent. toward the sun and 26.6 per cent. toward the initial position of the head. The low per cent of movements toward the water is too low to warrant the conclusion that the group, as a whole, is directed, in its movements, by the nearest body of water. However, certain individuals are undoubtedly so influenced. Two individuals made 100

TABLE VII.

WATER-STRIDER (*Gerris remigis* Say).

Specimen Number	Number of Trials	Age.	Kind of Water	Initial Positions.	Per Cent. of Movements.					
					Toward Water	Not Toward Water	Toward Sun.	Not Toward Sun.	Way Head Pointed.	Not Way Head Pointed.
1	10	Adt.	Br.	3	80	20	0	100	30	70
2	5	Adt.	Br.	4	40	60	0	100	40	60
3	1	Juv.	Br.	1	0	100	0	100	0	100
4	12	Juv.	Br.	8	33	67	1	99	50	50
5	2	Adt.	Br.	2	0	100	0	100	0	100
6	11	Juv.	Br.	5	9	91	0	100	27	73
7	11	Adt.	Br.	6	9	91	0	100	27	73
8	4	Adt.	Br.	1	25	75	50	50	0	100
9	2	Adt.	Br.	1	100	0	0	100	0	100
10	12	Adt.	Br.	3	0	100	50	50	0	100
11	2	Adt.	Br.	1	50	50	50	50	0	100
12	20	Juv.	Br.	4	30	70	25	75	50	50
13	1	Juv.	Br.	1	100	0	0	100	0	100
14	18	Adt.	Br.	3	11	89	89	11	17	83
15	9	Adt.	Br.	2	44	56	22	78	22	78
16	20	Adt.	Br.	4	20	80	50	50	50	50
17	6	Adt.	Lake	2	50	50	17	83	0	100
18	28	Adt.	Lake	6	36	64	14	86	61	39
19	40	Adt.	Lake	8	43	57	0	100	33	67
20	40	Adt.	Lake	8	25	75	7	93	52	48
21	40	Adt.	Lake	8	52	48	3	97	35	65
22	40	Adt.	Lake	8	18	82	2	98	40	60
23	40	Adt.	Lake	8	57	43	25	75	32	68
24	40	Adt.	Lake	8	32	68	5	95	40	60
25	40	Adt.	Lake	8	17	83	8	92	33	67
26	40	Adt.	Lake	8	30	70	7	93	42	58
27	40	Adt.	Lake	8	25	75	8	92	35	65
Total	534	Average.....		5	34.7	65.3	16	84	26.6	73.4

per cent. of movements toward the water; one, 80 per cent; four, between 50 and 60 per cent. On the other hand it must be noted that three individuals made 100 per cent. of their movements

away from the water; three, between 90 and 100 per cent.; four, between 80 and 90 per cent.; five, between 70 and 80 per cent.; four, between 60 and 70 per cent.; four, between 50 and 60 per cent. Evidently, although some individuals are undoubtedly influenced by water as a directive force, this is certainly not true of the group as a whole.

Near a small pond in Carondelet Park, St. Louis, Mo., there is a narrow valley. The floor, which is nearly horizontal is about 60 feet long and 20 feet wide. About eight feet from the pond it slopes, at an angle of 60 degrees to the water. On the two sides the walls slope upward at an angle of from 30 to 50 degrees. This valley floor was selected as the place in which to perform the following experiments with water-striders which had been captured in a brook near Creve Coeur Lake, Mo. One at a time the water-striders were placed on the ground, at a certain definite distance from the water. They were always faced away from the water. They were watched continuously until they had either reached the water or had gone from the starting point, in some direction other than toward the water, a distance equal to that from the starting place to the water. Such individuals were recorded as not moving toward the water. Sometimes an individual would start in a certain direction and continue in that direction to the close of the experiment. More often it wandered about in various directions before settling down to continue in one course. Sometimes an individual would move toward the water a distance of ten to twenty feet and then turn about and move away from it. In a few cases an individual would move away from the water a distance of ten to fifteen feet and then turn, meander about, and finally reach the water. Several of those that did not reach the water climbed the sides of the valley for a distance of fifteen to twenty feet. If, in its wanderings, an individual happened to arrive on the steep slope that extended from the horizontal floor of the valley to the pond, it invariably continued, sometimes with accelerated speed, to the water. The results are recorded in Table VIII.

These results harmonize with the conclusions based upon experiments with the checkerboard plate.

TABLE VIII.

Number of Individuals Used.	Number of Feet Placed From Water.	No. that Moved.		Per Cent that Moved.	
		To the Water.	Not Toward the Water.	To the Water.	Not Toward the Water.
20	10	6	14	30	70
10	20	2	8	20	80
10	30	2	8	20	80

THE WHIRLIGIG BEETLE (*Gyrinus* sp.?) (PLATE VII).

In working with the checkerboard plate twenty-one individuals were used. With the board from ten to fifteen feet from the water, 551 experiments were performed. The results are recorded in Table IX.

In an infinite series of experiments, if the factors considered have no effect of the movements of the creatures, then 25 per cent. of the movements should have been toward water; 12.5 per cent., toward the sun; 12.5 per cent. toward the original position of the head. These experiments yielded 52.6 per cent. of movements toward the water, 6.7 per cent. toward the position of the sun and 22 per cent. toward the original position of the head. In one individual the per cent. of movements toward the water was 95, in four cases it was between 80 and 90, in three cases it was between 60 and 70, in four cases it was between 50 and 60, in five it was between 40 and 50. In the case of only three individuals was the per cent. of movements toward water less than 30. Evidently, the whirligig beetle, when moving on the checkerboard plate, is largely directed by the nearest body of water. They act as though they were positively hydrotropic.

In addition to the work upon the checkerboard plate, the following experiments were conducted in the open. Twelve specimens were placed in a dry tray ten feet from the water. In less than half an hour all had flown to the water. Ten specimens were placed in a dry tray twenty feet from the water. In less than half an hour all had flown to the water. Ten specimens

TABLE IX.

THE WHIRLIGIG BEETLE (*Gyrinus* sp. ?.)—ADULTS.

Specimen Number.	Number of Trials.	Kind of Water.	Initial Positions.	Per Cent. of Final Movements.						Time of Day.
				Toward Water.	Not Toward Water.	Toward Sun.	Not Toward Sun.	Way Head Pointed.	Not Way Head Pointed.	
1	5	Pond	1	80	20	0	100	0	100	A.M.
2	35	Pond	7	60	40	6	94	6	94	A.M.
3	17	Pond	4	88	12	0	100	29	71	A.M.
4	2	Pond	1	0	100	0	100	100	0	A.M.
5	9	Pond	2	11	89	0	100	100	0	A.M.
6	5	Pond	1	0	100	0	100	20	80	A.M.
7	40	River	8	45	55	7	93	27	73	A.M.
8	5	River	1	40	60	0	100	0	100	A.M.
9	40	River	8	55	45	15	85	13	87	A.M.
10	17	River	3	37	63	5	95	12	88	P.M.
11	40	River	8	87	13	0	100	13	87	A.M.
12	41	River	8	56	44	0	100	20	80	A.M.
13	13	River	3	77	23	0	100	8	92	A.M.
14	40	River	8	30	70	15	85	15	85	A.M.
15	40	River	8	88	12	3	97	0	100	P.M.
16	41	River	8	68	32	30	70	17	83	P.M.
17	41	River	8	58	42	2	98	36	64	P.M.
18	40	River	8	43	57	15	85	15	85	P.M.
19	40	River	8	42	58	13	87	7	93	P.M.
20	20	River	4	95	5	0	100	20	80	P.M.
21	20	River	4	45	55	30	70	5	95	P.M.
Total	551	Av...	5.2	52.6	47.4	6.7	93.3	22	78	

were placed in a dry tray forty feet from the water. In less than half an hour all had flown to the water. In most cases they did not ascend high in the air; but the further back they were the higher they ascended. In one case a sidewise flight carried the beetle behind a tall tree. In that case it arose to above the top of the tree before turning toward the water.

At 3:15 P.M. the eyes of ten individuals were blinded by painting them with lampblack mixed in liquid glue. These whirligigs were placed in a dry tray forty feet from the water. Ten normal individuals were placed in a similar tray, at the same distance from the water. The two trays were only two feet apart. One by one, the individuals with normal sight arose from the tray and flew to the water. By 3:30 P.M. all but one had reached the water. At 4:00 P.M. the last one flew to the water. Up to

4:15 P.M., when I stopped for the day, none of the blinded whirligigs had reached the water. Two had become lost in the grass. Eight were still in the tray.

These experiments show conclusively that the nearest body of water exerts a directive influence upon the movements of the whirligig beetle and that the control is exerted by means of stimulus received through the eyes.

Conclusions

1. The checkerboard plate consists of a smooth board covered with white oilcloth which is subdivided, by printed lines, into one-inch squares. There is a leveling device by means of which it can be made perfectly horizontal. Considering the center of the board the center of a circle, the board is divided into eight sections, each forty-five degrees wide. Starting at the middle of one side the tips of these dividing lines are numbered from 0 to 315. This nomenclature makes it possible to arrange the specimen at the center of the board, with its head pointing in a certain definite direction. When in use, by means of an adjustable screen, the board is protected from the direct rays of the sun. It is washed from time to time to free it from odors that may influence movements. Thus manipulated all possibility of geotactic, chemotactic, and thigmotactic responses are eliminated; hence this device forms an excellent means of investigating the influence of bodies of water upon the behavior of small animals.

1. It may be used in studying any walking land insect, milliped, centiped, crustacean, or annelid.

2. It may be used in studying flying insects that also walk; but, in some cases it may be necessary to clip the wings.

3. It may be used in investigating the behavior of many fresh-water invertebrates (small snails, amphipods, isopods, crayfish, some backswimmers, giant water bugs, water-striders, larvæ of straight-winged flies, dragonfly larvæ, scavenger beetles, diving beetles, whirligig beetles); in some cases it cannot be used because the animals cannot remain out of water long enough (damsel-fly larvæ); in yet others it cannot be used because the creatures are too awkward when out of the water (large snails, water-tigers).

4. Although the matter has not been tested, it should be possible to use it in investigating many marine invertebrates.

5. This device may be used, not only with creatures with sound vision; but, also, with forms that have been rendered blind by manipulation.

II. In testing this device several ecological types of invertebrates were used: those that creep along the bottom of the body of water or along water plants, but do not leave the pond or stream (*Asellus*, half grown dragonfly larvæ, pond snails [these occasionally venture beyond the water edge]); those that creep along the bottom or up vegetation and occasionally leave the water (crayfish, scavenger beetles); those that live on the film and never depart far from it (giant water striders); those that dwell on the film, but migrate from one body of water to another (whirligig beetles).

1. The movements of neither pond snails, asellids nor dragonfly larvæ are influenced by nearby bodies of water.

2. In the majority of cases the movements of crayfish are controlled by the nearest body of water. When out of the water they behave as though they are positively hydrotropic. Blind crayfish do not so react; this demonstrates that the movements of this crustacean, when out of the water, are controlled by visual stimuli furnished by the nearest body of water.

3. About half of the scavenger beetles investigated act as though they are positively hydrotropic; the other half are not influenced, in a directive manner, by nearby bodies of water.

4. When on land, the majority of the water striders investigated are not influenced, in their movements, by the nearest body of water; a few act as though they are positively hydrotropic.

5. When out of the water, whirligig beetles are undoubtedly influenced, in their movements, by the nearest body of water. On the checkerboard plate the majority always move toward the water; when set at liberty within forty feet of a body of water, they always fly toward it. If blinded they do not react in this manner. This demonstrates that they are controlled by visual stimuli furnished by the water.

6. This paper does not contend that these movements which some of the forms make toward water are positive hydrotropisms

in the Loebian sense. These investigations prove that the movements mentioned are caused by the directive power of some body of water and not by gravity, nor the sun's rays, nor contact stimuli, nor odor trails. As to whether they are responses to sensations or are merely tropisms it has nothing to say. Either interpretation seems equally plausible.

7. Although the board was protected from the direct rays of the sun, almost all of the species used exhibited a marked tendency to move away from that portion of the board which was nearest the position of the sun. Since the sun's rays did not impinge on the individuals, this avoidance cannot be a negative phototropism. It must be a form of differential sensitivity. Perhaps it should be called negative photosensitivity.

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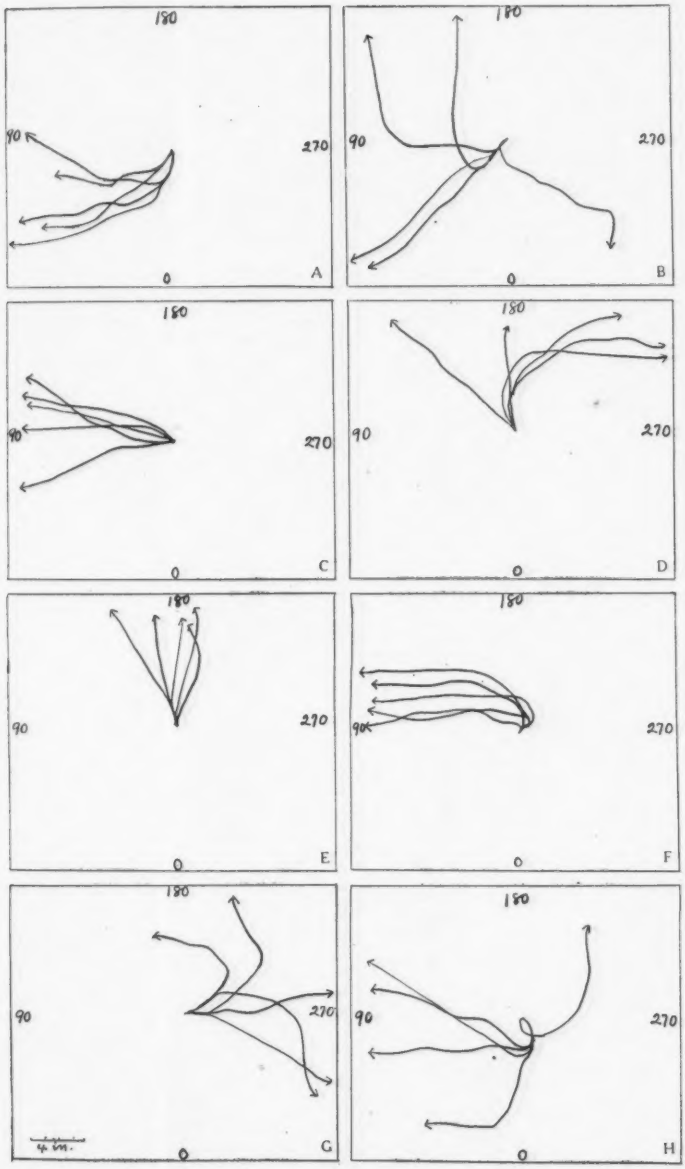

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PLATE I. DRAGON FLY NYMPHS, SERIES OF 40 TRIALS.

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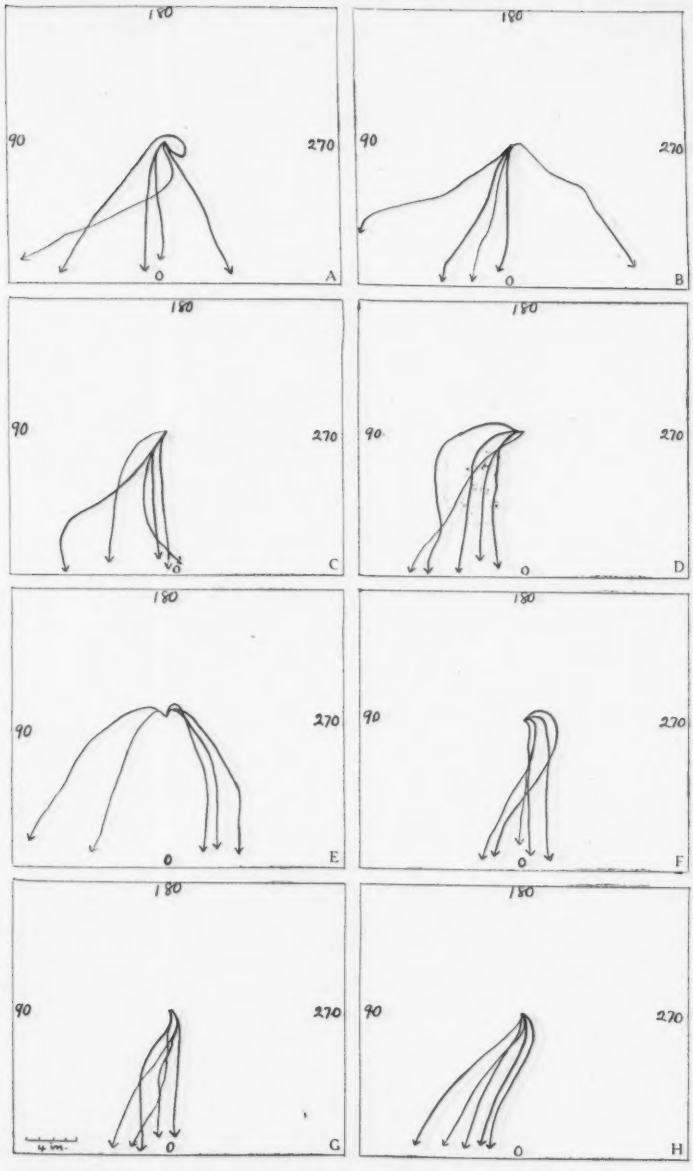

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PLATE II. NORMAL CRAYFISH.

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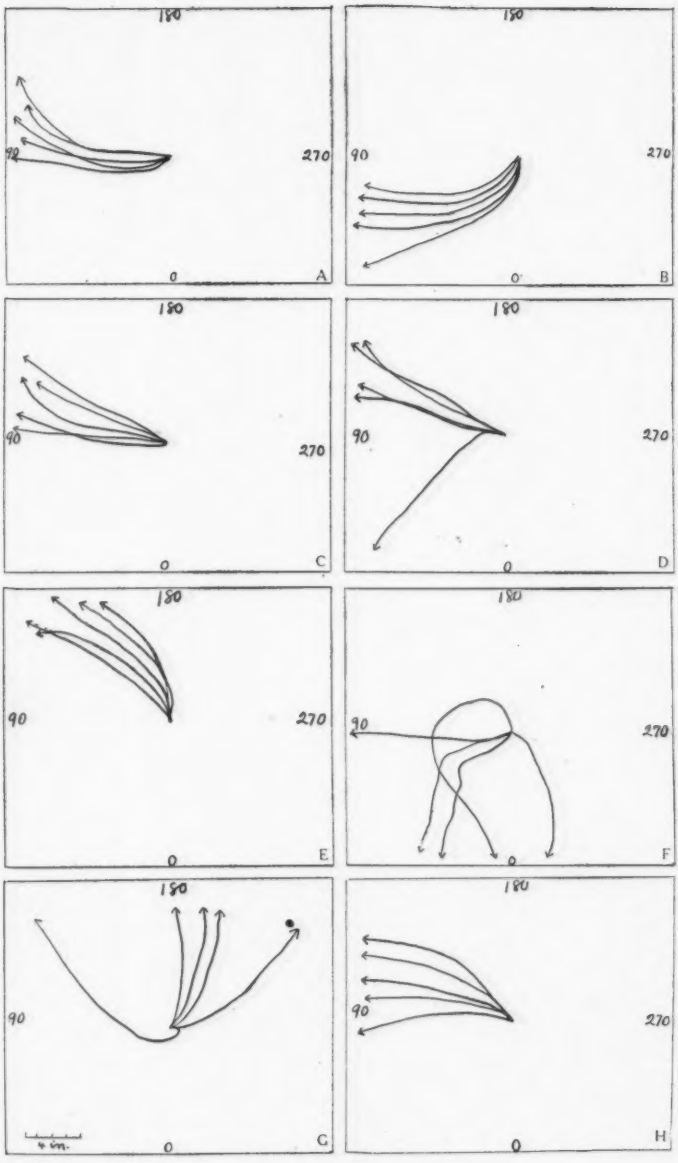

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PLATE III. BLINDED CRAYFISH.

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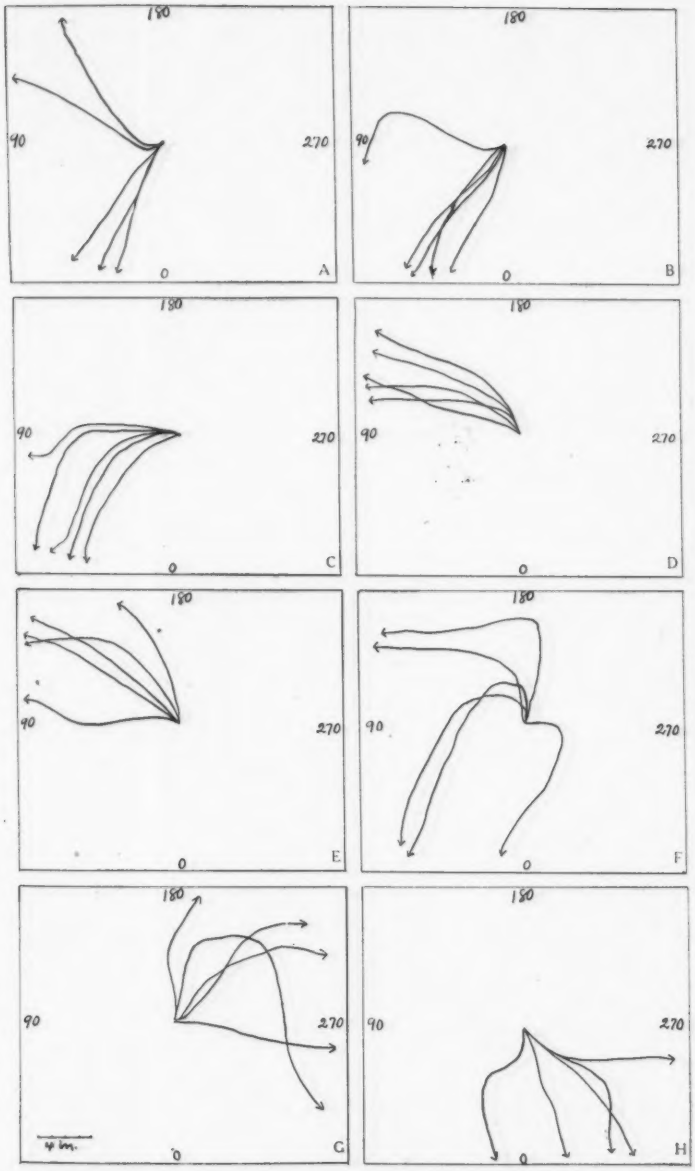

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PLATE IV. GIANT WATER BUG.

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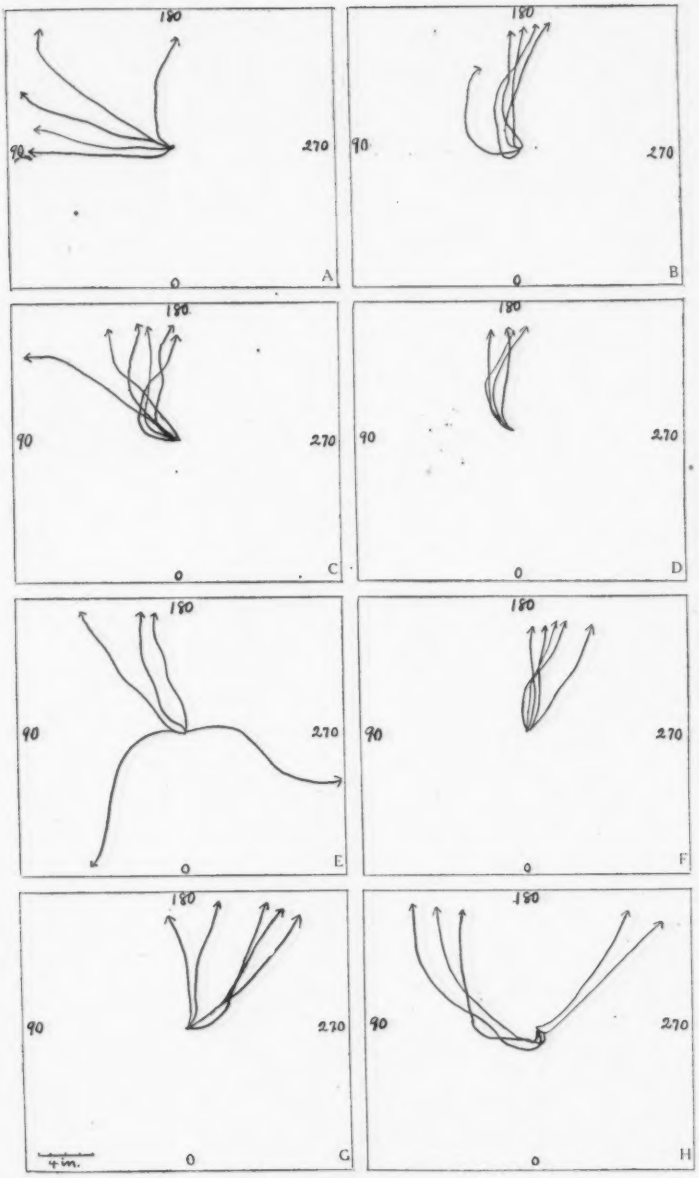

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PLATE V. SCAVENGER BEETLE.

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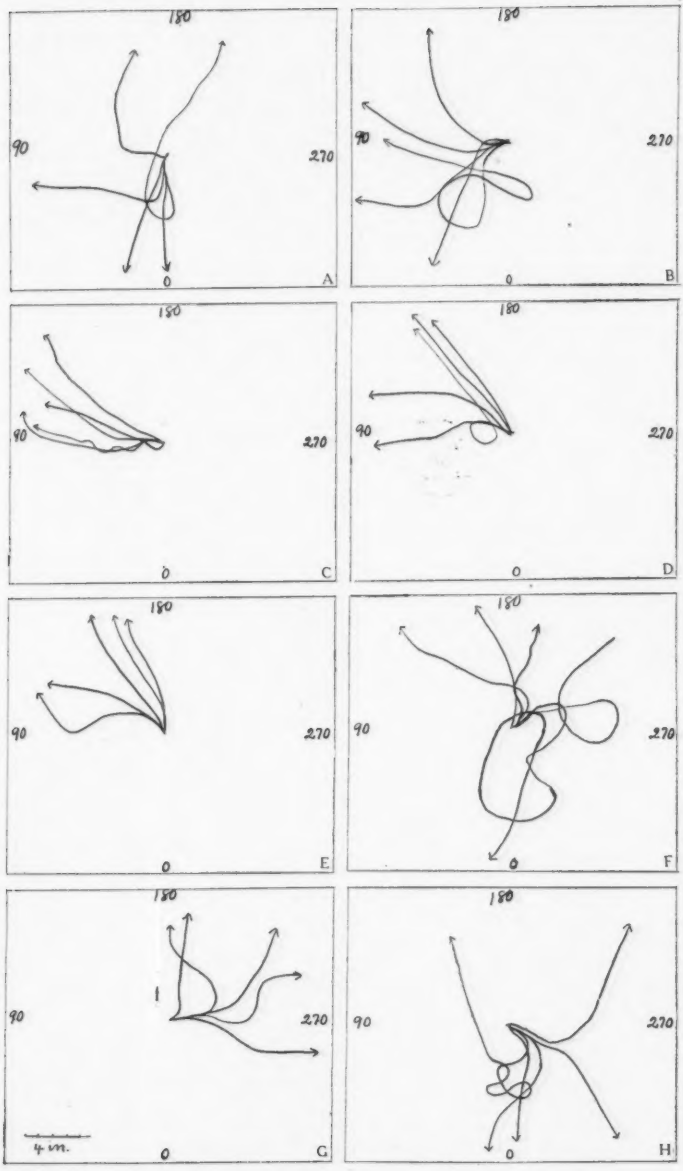

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PLATE VI. LARGE WATER STRIDER.

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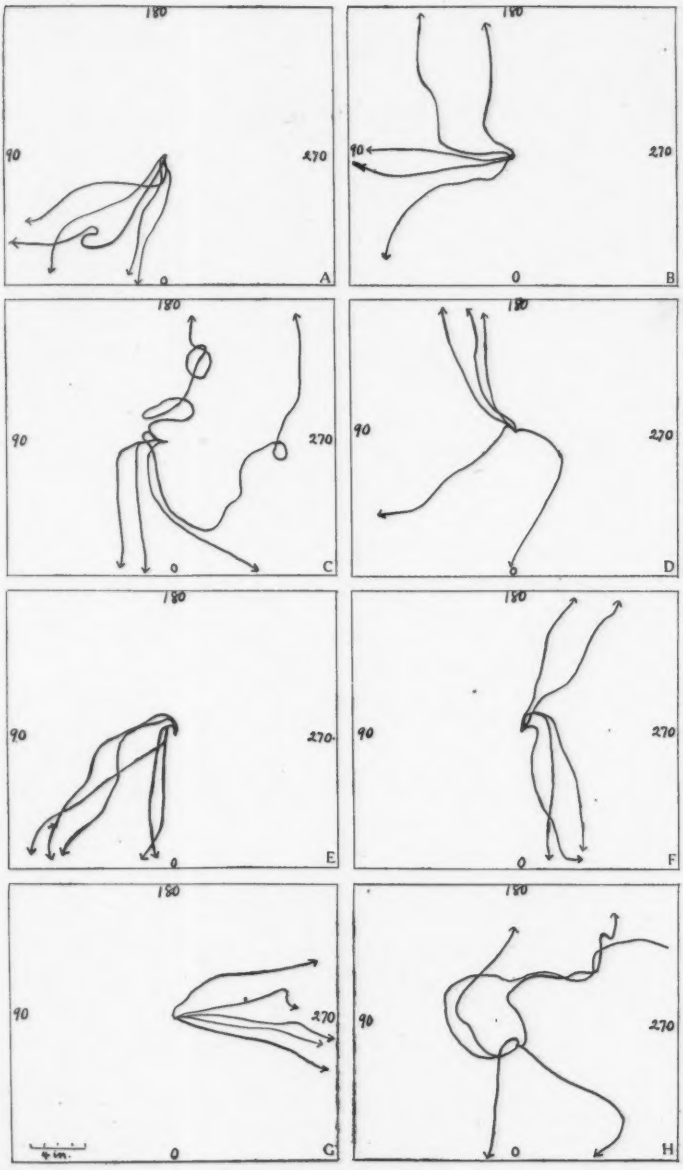

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PLATE VII. THE WHIRLIGIG BEETLE.

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